ENDOCRINOLOGY

VOLUME 54

JANUARY-JUNE 1954

EDITORIAL BOARD

A. ALBERT J. T. BRADBURY ANNE C. CARTER

L. L. ENGEL J. W. EVERETT R. GAUNT

N. S. HALMI R. HERTZ D. J. INGLE

L. P. LEBLOND J. MEITES

ROY O. GREEP, *Managing Editor*

25 Shattuck St., Boston 15, Massachusetts

PUBLISHED FOR
THE ENDOCRINE SOCIETY

Hospital Library

610.5
E564
v.54

Copyright, 1954, by the Association for the
Study of Internal Secretions, Inc. All rights reserved.

George Banta Publishing Company
Menasha, Wisconsin

Hospital

CONTENTS OF VOLUME 54

NO. 1, JANUARY, 1954

HOEXTER, FRIEDA M.
The Effect of Thyroidectomy on the O_2 Uptake of Brain Cortex and Liver in the Rat 1

LEE, NORMAN D. AND ROBERT H. WILLIAMS
The Intracellular Localiztion of Labeled Thyroxine and Labeled Insulin in Mammalian Liver 5

MANSOUR, TAG E. AND WILLIAM F. HEWITT, JR.
Sensitivity to Insulin During Adrenocortical Response to Cold Stress in Rats 20

SILBERBERG, MARTIN, RUTH SILBERBERG AND MARION OPDYKE
Degenerative Joint Disease in Mice Bearing Anterior Hypophyseal, Ovarian and Adrenal Grafts 26

WOLLMAN, SEYMOUR H.
A Thyroid Model Describing Kinetics of Exchange, Concentrating, and Organic Binding of Iodide 35

CAMPBELL, JAMES, L. CHAIKOFF AND I. W. F. DAVIDSON
Metahypophyseal Diabetes Produced by Growth Hormone 48

SMITH, THOMAS C.
The Action of Relaxin on Mammary Gland Growth in the Rat 59

DUMM, MARY E. AND ELAINE P. RALLI
A Critical Analysis of the Eosinophile Response in Rats to ACTH and Cortisone. 71

COHEN, HERMAN, HENRY A. FREEDMAN, WILLIAM KLEINBERG, NORMAN R. SCHECHTER AND GUSTAV J. MARTIN
Enhancement of Corticotrophic Activity: Effect on Adrenal and Thymus Weights 81

CRAFTS, ROGER C.
The Prevention of Anemia in Hypophysectomized Adult Female Rats with Combine Thyroxine and Cortisone Therapy 84

EISENBERG, EUGENE AND GILBERT S. GORDAN
The Effect of Growth-Inhibiting Conditions upon the Levator Ani Muscle of the Rat 93

HALMI, N. S.
Thyroidal Iodide Trapping as Influenced by Serum Iodide Levels and Thyrotrophin 97

LEBLOND, C. P.
Chemical Form of the Iodine Present in the Hair of the Rat 104

NOTES AND COMMENTS
KOCSIS, J. J. AND L. G. ABOOD, 108; MEYER, ARTHUR E., 110; MITCHELL, M. L., R. GUILLEMIN AND H. SELYE, 111; KITAY, JULIAN I., 114; BASTIAN, W. J. AND M. X. ZARROW, 116

THE ENDOCRINE SOCIETY
The 1954 Annual Meeting, 118; 1954 Awards and Fellowships, 119

NO. 2, FEBRUARY, 1954

WILLIAMS-ASHMAN, H. G.
Changes in the Enzymatic Constitution of the Ventral Prostate Gland Induced by Androgenic Hormones 121

SANTISTEBAN, GEORGE A. AND THOMAS F. DOUGHERTY
Comparison of the Influences of Adrenocortical Hormones on the Growth and Involution of Lymphatic Organs 130

HOELSCHER, BERNHARD AND KURT R. REISSMANN
The Combined Effects of Hypoxia, Adrenalectomy and Thymectomy in Parabiotic Rats 147

NADLER, N. J., C. P. LEBLOND AND R. BOGOROCH
The Rate of Iodine Metabolism by the Thyroid Follicle as a Function of Its Size. 154

WESTERMEYER, V. W. AND M. S. RABEN
Fall in Blood Sugar from Anterior Pituitary Extract 173

GOLDBERG, R. C. AND J. WOLFF
Evaluation of the Antithyroid Activity of 5-Iodo-2-Thiouracil 181

JOHNSON, BEN B.
Bioassay of Adrenal Cortical Steroids on the Basis of Electrolyte Excretion by Rats: Effects of 11-Desoxy and 11-Oxy-Steroids 196

HEARD, R. D. H. AND V. J. O'DONNELL
Biogenesis of the Estrogens: The Failure of Cholesterol-4-C^{14} to Give Rise to Estrone in the Pregnant Mare 209
HALMI, N. S.
Regulation of the Rat Thyroid in Short-term Iodine Deficiency 216
NOTES AND COMMENTS
KORSON, ROY AND A. L. BOTKIN
Effects of Total Body X-Irradiation on the Rat Pituitary 225
FLEISCHMANN, WALTER AND JOHN T. BRACKIN, JR.
Weight Response of Irradiated Accessory Sex Organs of the Castrate Male Rat to Testosterone Propionate 227
HERTZ, ROY, WILLIAM TULLNER AND EDWARD RAFFELT
Progestational Activity of Orally Administered 17α-Ethinyl-19-Nortestosterone 228
SHELLABARGER, C. J. AND J. T. GODWIN
Effects of Triiodothyronine on Tadpoles 230
RISS, WALTER AND WILLIAM C. YOUNG
The Future of Large Quantities of Testosterone Propionate to Activate Low Drive Male Guinea Pigs 232
THE ENDOCRINE SOCIETY
The 1954 Annual Meeting 236
1954 Award and Fellowships 237
THE AMERICAN GOITER ASSOCIATION 1954 ANNUAL MEETING 238

NO. 3, MARCH, 1954

CROXATTO, H., L. BARNAFI, L. CAMAZON AND V. PARRA
Some Endocrine Factors in the Polyuric Action of Renin 239
EARTLY, H. AND C. P. LEBLOND
Identification of the Effects of Thyroxine Mediated by the Hypophysis 249
GAUNT, ROBERT, J. H. LEATHEM, CONSTANCE H. TUTHILL, NANCY ANTONCHAK, MARTHA GILMAN AND A. A. RENZI
Some Biological Properties of Aminosteroids 272
ELLIOTT, J. R. AND C. W. TURNER
The Mammary Gland Spreading Factor in Normal Pregnant Animals 284
LINK, R. P. AND L. E. ST. CLAIR
Some Metabolic Studies on Hypophysectomized Pigs 290
SCHAFFENBURG, C. A. AND E. PERRY MCCULLAGH
Studies in Sperm Hormones: Demonstration of Estrogenic Activity 296
FODDEN, JOHN H. AND WILLARD O. READ
The Activity of Extracted Pancreatic Hyperglycemic-Glycogenolytic Factor After Cobaltous Chloride and Synthalin A 303
RICHTER, CURT P. AND E. H. UHLENHUTH
Comparison of the Effects of Gonadectomy on Spontaneous Activity of Wild and Domesticated Norway Rats 311
BEYLER, A. L. AND C. M. SZEGO
Correlation of Ovarian Cholesterol Levels with Changes in β-Glucuronidase Activity of Reproductive Tract During the Estrous Cycle and Pregnancy 323
BEYLER, A. L. AND C. M. SZEGO
Steroid Hormone Interactions as Evidenced by Modification of β-Glucuronidase Activity of the Preputial Glands of the Rat 334
HELMER, O. M. AND MARY ROOT
The Effect of ACTH and Cortisone on the Hyperglycemic Response to Glucagon 338
BRADLOW, H. LEON, KONRAD DOBRINER AND T. E. GALLAGHER
The Fate of Cortisone-*T* in Mice 343
NOTES AND COMMENTS
COHEN, SAUL L., M. M. GOLDFINE, FLORENCE TOUSSAINT, KAROL FRIEDMAN AND IRIS NOMA
The Hydrolysis of Pregnanediol Glucuronide by Glucuronidase Preparations. 353
LADMAN, AARON J. AND RUSSELL J. BARRNETT
Histochemical Demonstration of Protein-Bound Sulfhydryl and Disulfide Groups in Cells of the Anterior Pituitary 355
THE ENDOCRINE SOCIETY
The 1954 Annual Meeting 361
1954 Award and Fellowships 361

NO. 4, APRIL, 1954

ROSEMBERG, EUGENIA, JEROME CORNFIELD, ROBERT W. BATES AND EVELYN ANDERSON
Bioassay of Adrenal Steroids in Blood and Urine Based on Eosinophil Response: A Statistical Analysis 363
DAVIS, M. EDWARD AND ERNST JURGEN PLOTZ
The Effects of Cortisone Acetate on Intact and Adrenalectomized Rats During Pregnancy 384
SHELESNYAK, M. C.
Comparative Effectiveness of Antihistamines in Suppression of the Decidual Cell Reaction in the Pseudopregnant Rat 396
BACCHUS, HABEEB
Maintenance of Blood 17-Hydroxycorticosteroids Following Ascorbic Acid Treatment 402
HISAW, FREDERICK L. AND JOSEPH T. VELARDO
Influence of a Potentiator of Estrogen in the Inhibition of Progesterone in Development of Deciduomata 409
TABER, ELSIE AND KATHRYN W. SALLEY
The Effects of Sex Hormones on the Development of the Right Gonad in Female Fowl 415
LANGLEY, L. L., P. W. SCOKEL AND E. J. MOORE
Role of Hypoglycemia and Carbon Dioxide in the Reaction to Stress 425
MILLER, ZELMA
A Study of the Lymphocytolic Action of an Adrenal Cortical Extract *in Vitro* .. 431
VANDERLAAN, W. P. AND RAYNOR CAPLAN
Observations on a Relationship Between Total Thyroid Iodine Content and the Iodide-Concentrating Mechanism of the Thyroid Gland of the Rat 437
CLEGG, M. T., J. M. BODA AND H. H. COLE
The Endometrial Cups and Allantochorionic Pouches in the Mare with Emphasis on the Source of Equine Gonadotrophin 448
JANES, RALPH G.
Effect of a Pituitary Gonadotrophin on the Ovaries of Hypothyroid Rats 464
NOTES AND COMMENTS
DICZFALUSY, EGON
Poly-Estradiol Phosphate (PEP); a Long-Acting Water Soluble Estrogen .. 471
ESSELLIER, A. F., P. JEANNERET, E. KOPP AND L. MORANDI
Evidence Against Destruction of Eosinophils by Glucocorticoids as Shown by *In Vitro* Experiments 477
THE ENDOCRINE SOCIETY
The 1954 Annual Meeting 480
THE LAURENTIAN HORMONE CONFERENCE
1954 Annual Meeting 480
BOOK REVIEW 482

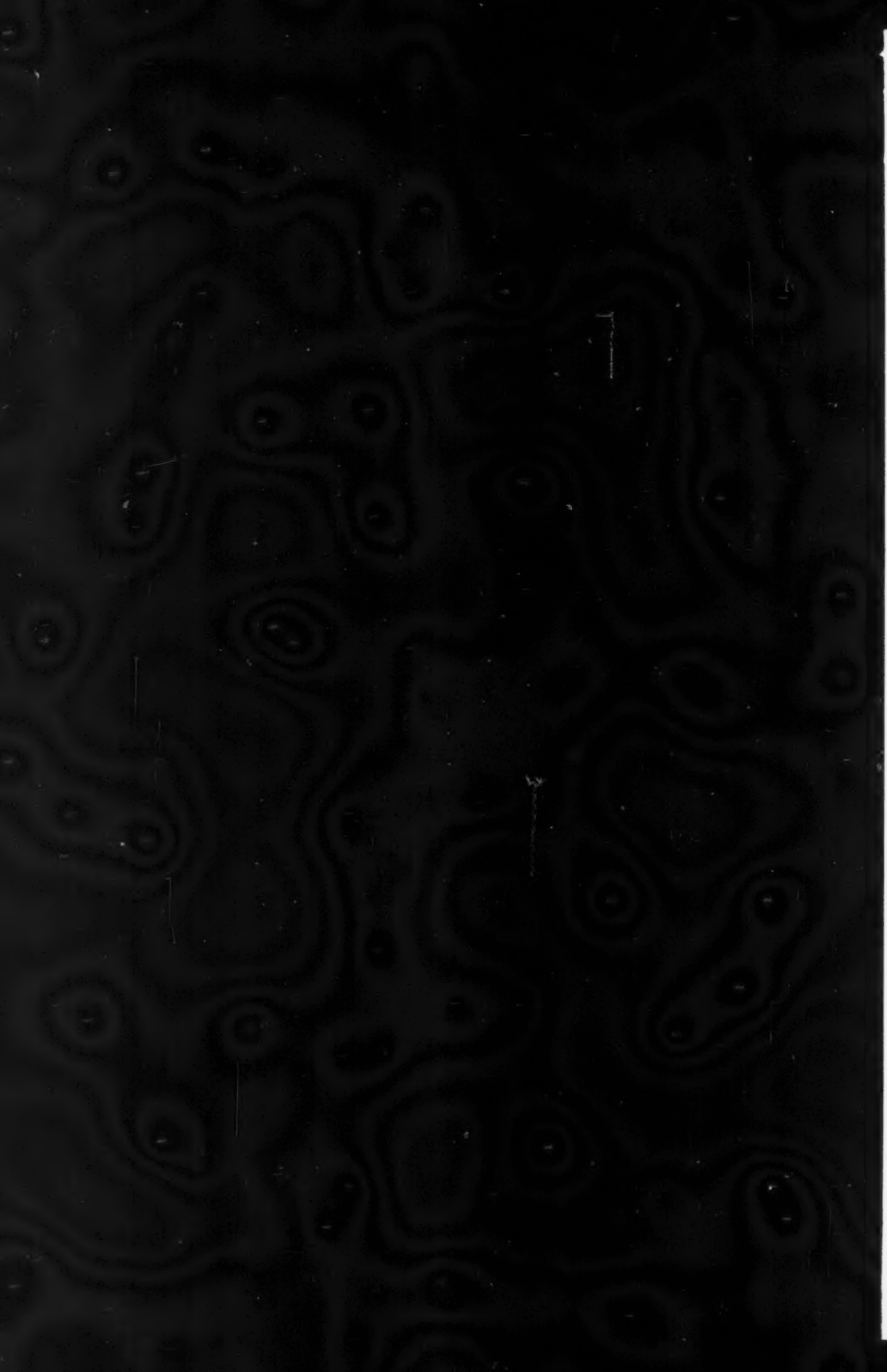
NO. 5, MAY, 1954

RABASA, S. L., FRIDA BERGMANN AND A. D. DOMÉNICO
Effect of DCA and NaCl Loading on Aminopterin Intoxication and Weight of Thymus 483
MIRSKY, I. ARTHUR, MARVIN STEIN AND GERHARD PAULISCH
The Secretion of an Antidiuretic Substance into the Circulation of Rats Exposed to Noxious Stimuli 491
ENGFELDT, BENGT AND ROLF ZETTERSTRÖM
Biophysical and Chemical Investigation on Bone Tissue in Experimental Hyperparathyroidism 506
FARQUHAR, MARILYN GIST AND JAMES F. RINEHART
Electron Microscopic Studies of the Anterior Pituitary Gland of Castrate Rats .. 516
CRAFTS, ROGER C.
The Value of a High Protein Diet in the Prevention of Anemia in Hypophysectomized Adult Female Rats by Means of Thyroxine and Androgen Therapy 542
HOUSSAY, B. A., R. R. RODRIGUEZ AND A. F. CARDEZA
Prevention of Experimental Diabetes with Adrenal Steroids 550
SHIZUME, KAZUO, AARON BUNSEN LERNER AND THOMAS B. FITZPATRICK
In Vitro Bioassay for the Melanocyte Stimulating Hormone 553
STURTEVANT, F. M. AND NANCY FULLER
A Simplified Test for Anti-Diabetic Activity 561

D'Angelo, S. A., C. E. Stevens, K. E. Paschkis, A. Cantarow, F. William Sunderman and G. Friedler
The Effect of Goitrogen Withdrawal on the Pituitary-Thyroid System of the Guinea Pig 565
Hofmann, Frederick G. and Clarke Davison
Observations on *in Vitro* Adrenal Steroid Synthesis in the Rat 580
Bacila, Metry and E. S. Guzman Barron
The Effect of Adrenal Cortical Hormones on the Anaerobic Glycolysis and Hexokinase Activity 591
The Endocrine Society
The 1954 Annual Meeting 604

NO. 6, JUNE, 1954

van Tienhoven, A., A. V. Nalbanov and H. W. Norton
Effect of Dibenamine on Progesterone-Induced and "Spontaneous" Ovulation in the Hen 605
Venkataraman, Poonamalle R. and Morris T. Friedell
The Effects of Induced Hyperthyroidism and Vitamin B_{12} on the Synthesis of Some Phosphate Fractions in the Rat Liver 612
Thompson, James C. and Raymond F. Blount
The Age of Beginning Reactivity of the Pituitary-Adrenocortical System to Stress in the Mouse 620
Josimovich, John B., Aaron J. Ladman and Helen Wendler Deane
A Histophysiological Study of the Developing Adrenal Cortex of the Rat During Fetal and Early Postnatal Stages 627
Cavazos, L. F. and R. M. Melampy
Cytological Effects of Testosterone Propionate on Epithelium of Rat Seminal Vesicles 640
Torda, Clara and Harold G. Wolff
Effects of Various Hormones on the Amounts of Phosphorus Containing Fractions of the Brain 649
Davison, Clarke and Frederick G. Hofmann
Influence of Sulfhydryl Compounds on *In Vitro* Steroid Production by the Rat Adrenal Gland 654
Anderson, Bernhard G.
Potency and Duration of Action of Triiodothyronine and Thyroxine in Rats and Mice 659
Hoover, Charles R. and C. W. Turner
In Vitro Metabolism of Rat Mammary Gland: Respiration, Aerobic Glycolysis and Carbon Dioxide Productions 666
Bahn, Robert C. and David Glick
Studies in Histochemistry: Effects of Stress Conditions, ACTH, Cortisone and Desoxycorticosterone on the Quantitative Histological Distribution of Ascorbic Acid in Adrenal Glands of the Rat and Monkey 672
Everett, John W.
Luteotrophic Function of Autografts of the Rat Hypophysis 685
Mirsky, I. Arthur, Gerhard Paulisch and Marvin Stein
The Antidiuretic Activity of the Plasma of Adrenalectomized, Hypophysectomized and Adrenalectomized-Hypophysectomized Rats 691
Swingle, W. W., Max Ben, Robert Maxwell, Carleton Baker, Edward Fedor and George Barlow
Effect of the Sodium-retaining Factor of the Adrenal Cortex Upon the Serum Electrolytes of Adrenalectomized Dogs 698
Notes and Comments
Glick, David, Richard H. Swigart, Robert C. Bahn and Donald H. Hastings
A Technique for Killing Animals to Obtain Tissues for Studies on Stress Effects 706
Stoner, H. B. and D. Dexter
Glomerular Filtration Rate After Adrenal Medullectomy in the Rat 708
New Book Register 710
Index 711



OES NOT CIRCULATE

Volume 54 JANUARY, 1954 Number 1

ENDOCRINOLOGY

UNIV OF MI JAN 13 1954 HOSPITAL LIBRARY

HOEXTER, FRIEDA M.
The Effect of Thyroidectomy on the O_2 Uptake of Brain Cortex and Liver in the Rat 1

LEE, NORMAN D. AND ROBERT H. WILLIAMS
The Intracellular Localization of Labeled Thyroxine and Labeled Insulin in Mammalian Liver 5

MANSOUR, TAG E. AND WILLIAM F. HEWITT, JR.
Sensitivity to Insulin During Adrenocortical Response to Cold Stress in Rats 20

SILBERBERG, MARTIN, RUTH SILBERBERG AND MARION OPDYKE
Degenerative Joint Disease in Mice Bearing Anterior Hypophyseal, Ovarian and Adrenal Grafts 26

WOLLMAN, SEYMOUR H.
A Thyroid Model Describing Kinetics of Exchange, Concentrating, and Organic Binding of Iodide 35

CAMPBELL, JAMES, L. CHAIKOF AND I. W. F. DAVIDSON
Metahypophyseal Diabetes Produced by Growth Hormone 48

SMITH, THOMAS C.
The Action of Relaxin on Mammary Gland Growth in the Rat 59

DUMM, MARY E. AND ELAINE P. RALLI
A Critical Analysis of the Eosinophile Response in Rats to ACTH and Cortisone 71

COHEN, HERMAN, HENRY H. FREEDMAN, WILLIAM KLEINBERG, NORMAN R. SCHECHTER AND GUSTAV J. MARTIN
Enhancement of Corticotrophic Activity: Effect on Adrenal and Thymus Weights 81

CRAFTS, ROGER C.
The Prevention of Anemia in Hypophysectomized Adult Female Rats with Combined Thyroxine and Cortisone Therapy 84

EISENBERG, EUGENE AND GILBERT S. GORDAN
The Effect of Growth-Inhibiting Conditions upon the Levator Ani Muscle of the Rat 93

HALMI, N. S.
Thyroidal Iodide Trapping as Influenced by Serum Iodide Levels and Thyrotrophin 97

LEBLOND, C. P.
Chemical Form of the Iodine Present in the Hair of the Rat 104

NOTES AND COMMENTS
KOCSIS, J. J. AND L. G. ABOOD, 108; MEYER, ARTHUR E., 110; MITCHELL, M. L., R. GUILLEMIN AND H. SELYE, 111; KITAY, JULIAN I., 114; BASTIAN, W. J. and M. X. ZARROW, 116

THE ENDOCRINE SOCIETY
The 1954 Annual Meeting, 118; 1954 Awards and Fellowships, 119.

ISSUED MONTHLY FOR THE ENDOCRINE SOCIETY
BY CHARLES C THOMAS, PUBLISHER,
SPRINGFIELD, ILLINOIS

Annual Subscription $12.00 Single Copies $1.50

In female breast carcinoma

Results of a recent clinical study show that Neodrol is effective in the palliative treatment of advanced, inoperable breast cancer in the female. Of the 42 patients (some with both soft tissue and osseous metastases) treated with Neodrol, 43% demonstrated objective improvement.

OBJECTIVE IMPROVEMENT	with Neodrol	with testosterone or its esters
In soft tissue metastases	39% (14 of 37 pts.)	22% (38 of 174 pts.)
In osseous metastases	25% (8 of 32 pts.)	19% (26 of 133 pts.)

SUBJECTIVE IMPROVEMENT	Patients with referable symptoms	Patients improved with Neodrol
Pain	28	22
Anorexia	11	10
General Malaise	10	8
Cough	7	6
Dyspnea	13	8
Headache	6	6
Nausea	6	4
Vomiting	4	3
Total	36	31

Of the 36 patients with symptoms referable to their carcinoma, a total of 87% experienced symptomatic improvement under Neodrol therapy.

Escher, G. C., et al.: Clinical Research Proceedings 1:51 (Apr.) 1953.

with a NEW crystalline steroid

NEODROL*

BRAND OF STANOLONE

Supplied: 10 cc. vials, 50 mg./cc.

A PFIZER SYNTEX PRODUCT

Pfizer

PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc., Brooklyn 6, N.Y.

*TRADEMARK

ENDOCRINOLOGY

VOLUME 54 JANUARY, 1954 NUMBER 1

Copyright 1954 by the Endocrine Society

THE EFFECT OF THYROIDECTOMY ON THE O_2 UPTAKE OF BRAIN CORTEX AND LIVER IN THE RAT

FRIEDA M. HOEXTER*

Biochemical and Endocrinological Research Department, Bristol Mental Hospitals, Bristol, England

THE endocrines, in particular the thyroid, play an important role in brain function, but as yet knowledge of their inter-relationship is hardly more than speculative. Some investigations on the O_2 uptake of rat brain cortex and liver, after thyroidectomy, are reported here.

METHOD

Adult male Wistar rats were used. [Both normal control and operated animals were housed in a thermo-regulated room. Thyroid-parathyroidectomy was performed under a preparation microscope. The rats were sacrificed at weekly intervals after thyroidectomy, up to and including the sixth week. There was some loss of hair in the operated animals, usually apparent after the third week, but no marked changes in body weight.

The O_2 uptake of the tissue slices was determined by the Warburg method, at 37.0° C., using a Krebs Phosphate Buffer (pH 7.4) and glucose as substrate. The Q_{O_2} values are expressed as mm.^{3} O_2 per mg. dry weight per hour. Determinations on the brain cortex and liver of normal control and operated animals were carried out on the same day. Triplicate observations were made on each organ. In the case of liver, slices were always cut from the same lobe.

RESULTS

In Table 1, values for the O_2 consumption of brain cortex and liver of normal control rats are compared with the corresponding values for the operated rats. Mean values for at least five rats are calculated. No '*t*' test was necessary for the Q_{O_2} values of the brain cortex, since it is apparent that they are quite constant up to the sixth week after thyroidectomy. The

Received for publication April 6, 1953.

* Working under a Research Grant from Organon Laboratories, England.

TABLE 1. OXYGEN UPTAKE OF BRAIN CORTEX AND LIVER

Time after operation	Brain		Liver			
Days	No. of rats	Mean Q_{O_2} ± S.D.	No. of rats	Mean Q_{O_2} ± S.D.	*'t'*	*P*
0	25	8.57 ± 0.65	26	7.99 ± 1.14		
7	6	8.44 ± 0.68	6	7.64 ± 1.56	0.52	0.7 –0.5
14	6	8.45 ± 0.74	6	7.37 ± 1.88	0.65	0.7 –0.5
21	5	8.36 ± 0.39	5	7.12 ± 0.95	1.80	0.1 –0.05
28	5	8.67 ± 0.46	5	*6.72 ± 0.98*	*2.56*	0.02–0.01
35	5	8.67 ± 0.66	5	*6.27 ± 0.90*	*3.69*	>0.001
42	5	8.46 ± 0.55	5	*6.68 ± 1.24*	*2.18*	0.05–0.02

Legend: S.D. = Standard deviation or (variance)$^{1/2}$.
't' = Students *'t'* value.
P = Level of probability indicated by the *'t'* value.

Q_{O_2} values for liver, in both normal control and operated animals, were much more variable, as can be seen from the standard deviation values. There is a decrease in the liver Q_{O_2} values after thyroidectomy, which becomes significant after 4–6 weeks. The values for the liver weight, calculated per 100 gms. of body weight, are recorded in Table 2. These values follow the same pattern as the liver Q_{O_2} values, and there is a decrease which is significant after 4–6 weeks. The livers at this time presented a fatty appearance. It is worth noting that the brain cortex slices of the normal control rats were able to respire at a practically constant rate for at least three hours and that this constancy remained unaltered after thyroidectomy.

As the Q_{O_2} values for brain cortex remained so constant after thyroidectomy, a few further experiments were carried out to see whether ablation of adrenals or testes coupled with thyroidectomy would influence the O_2 uptake. The results were negative, as can be seen in Table 3. Neither additional adrenalectomy nor additional castration influenced the O_2 uptake of brain cortex in a significant way.

TABLE 2. LIVER WEIGHT/100 GM. BODY WEIGHT

Days after operation	No. of rats	Liver weight per 100 gm. body weight ± S.D.	*'t'*	*P*
0	22	3.61 ± 0.77		
7	5	3.14 ± 0.59	1.52	0.2 –0.1
14	4	3.61 ± 1.10	0.0	—
21	5	3.13 ± 0.27	1.17	0.3 –0.2
28	5	*2.87 ± 0.37*	*3.22*	0.01–0.001
35	5	*2.51 ± 0.50*	*3.93*	>0.001
42	5	*2.85 ± 0.61*	*2.38*	0.05–0.02

Legend: S.D. = Standard deviation or (variance)$^{1/2}$.
t = Students *'t'* value.
P = Level of probability indicated by the *'t'* value.

TABLE 3. INVESTIGATIONS AFTER FURTHER OPERATIVE PROCEDURES

First operation	No. of days between operations	Second operation	No. of days between final operation and experiment	No. of rats	$Q_{O_2} \pm$S.D.
—		—		25	8.57 ± 0.65
Adrenalectomy			3	2	8.14 ± 0.125
Castration			6	2	8.61 ± 0.195
Thyroidectomy	40–90	Castration	3–28	5	8.31 ± 0.38
Thyroidectomy	40–80	Adrenalectomy	3	3	8.39 ± 0.155
Castration	40–90	Thyroidectomy	7–35	3	8.61 ± 0.36

Legend: S.D. = Standard deviation or $(\text{variance})^{1/2}$.

DISCUSSION

Several workers have shown that the O_2 uptake of brain cortex remains unaltered after various experimental procedures. Brophy and McEachern (1949) found that the brain cortex of thyroxine-treated rats maintained a normal uptake and that the livers of these animals showed a variable response. Barker and Klitgaard (1952) found that in addition to brain, the Q_{O_2} of slices of testis and spleen were not significantly changed one month after thyroidectomy, or by administration of thyroxine. Crimson and Field (1940) found no change in the O_2 uptake of brain cortex of adrenalectomised rats. Macleod and Reiss (1940) found no change after hypophysectomy. Sprites (1941) found no change in the oxygen uptake of brain cortex of thyrotoxic guinea-pigs, and Gordon and Heming (1944) showed that thyroid treatment had no effect on the oxygen uptake of rat brain cortex. Conflicting results were reported by Eisenberg, Gordan and Elliott (1949), who found that the respiration rate of brain suspensions of castrated rats was 32% above normal, and by Cohen and Gerard (1937), who found an increase in the O_2 uptake of brain mince in thyroid-treated rats.

As the O_2 consumption of brain cortex is a resultant of the various metabolic processes taking place in the tissue, its constancy in the rats can be explained in two ways; either that the various metabolic processes are unaltered, which is unlikely, or that there are changes in the single metabolic components which do not, however, alter the final balance. This would indicate that the equilibrium of the oxidative processes in the brain is better regulated than in other organs. It would be interesting to see whether brains of higher species react similarly.

The metabolic processes of the liver are influenced by thyroidectomy. Entenman, Chaikoff and Reichert (1948) found fatty livers in thyroidectomised dogs. As the O_2 uptake of the liver, in both normal control and thyroidectomised rats, is variable, it is difficult to evaluate the changes which occur after thyroidectomy. Here again the possibility of compensatory

mechanisms cannot be excluded. It would seem that the increase in glycogen content of the liver after thyroidectomy (De Minjer, 1948) is a more constant and pronounced effect than the fall in over-all O_2 uptake of the liver.

SUMMARY

The O_2 uptake of brain cortex and liver in normal control and thyroidectomised rats was investigated. The O_2 uptake of brain cortex remained unchanged 1–6 weeks after thyroidectomy. The O_2 uptake of the liver was influenced by thyroidectomy, and was significantly decreased after 4–6 weeks.

The effect on the O_2 uptake of brain cortex of adrenalectomy or castration coupled with thyroidectomy was investigated. Neither additional adrenalectomy nor additional castration influenced the O_2 uptake of brain cortex in a significant way.

Acknowledgment

The author is indebted to Dr. Max Reiss for his advice and interest in this work.

REFERENCES

Barker, S. B. and H. M. Klitgaard: *Am. J. Physiol.* **170:** 81. 1952.
Brophy, D. and D. McEachern: *Proc. Soc. Exp. Biol. & Med.* **70:** 120. 1949.
Cohen, R. A. and R. W. Gerard: *J. Cell. & Comp. Physiol.* **10:** 223. 1937.
Crimson, J. M. and J. Field: *Am. J. Phys.* **130:** 231. 1940.
De Minjer, A.: *Nederl. tijdsch. v. geneesk.* **4:** 3570, 1948.
Eisenberg, E., G. S. Gordan and H. W. Elliot: *Science.* **109:** 337. 1949.
Entenman, C., I. L. Chaikoff and F. L. Reichert: *Endocrinology* **42:** 210. 1948.
Gordon, E. C. and A. E. Heming: *Endocrinology* **34:** 353. 1944.
MacLeod, L. D. and M. Reiss: *Biochem. J.* **34:** 820. 1940.
Sprites, M. A.: *Proc. Soc. Exp. Biol. & Med.* **46:** 279. 1941.

THE INTRACELLULAR LOCALIZATION OF LABELED THYROXINE AND LABELED INSULIN IN MAMMALIAN LIVER[1]

NORMAN D. LEE[2] AND ROBERT H. WILLIAMS

Department of Medicine, University of Washington School of Medicine, Seattle, Washington

IMPLICIT in every endocrinological experiment is the concept of a specific site of hormone action. This concept may be expressed with varying degrees of precision and defined in either physiological or anatomical terms. It is a truism, however, that hormone action must be ultimately referable to the chemistry of the cell. When viewed in this manner the following questions assume fundamental importance: (1) do hormones penetrate the cell wall and enter the cytoplasm, and (2) if so, is their intracellular existence associated with cytological structures?

These questions have not been entirely ignored. The mere fact that hormones are secreted into the blood and may be found in the urine or intestinal contents is ample evidence for transmission across cell membranes. Such observations are of limited usefulness, however, with respect to the above questions and a paucity of information exists in this area. The investigations of Stadie, *et al.* (1949), Hills and Stadie (1952) and Haugaard and Marsh (1952) on insulin binding to tissue are notable contributions. However, a direct study of these questions has been confined solely to the work of Lipner, *et al.* (1952a, 1952b). The present report is concerned with the nature of the cellular penetration, cytoplasmic distribution and binding of I^{131}-labeled thyroxine and insulin in the liver of the adult female albino rat.

METHODS

1. *Isotopic Preparations*

L-Thyroxine-I^{131}[3], labeled in the 3′, 5′ positions, was used in doses of 0.09 or 6.0 μgm. and contained 1.6 to 5.0 μc. Insulin was labeled with I^{131} according to Ferrebee's procedure (1951), the starting material being crystalline zinc insulin.[4] The iodinated

Received for publication April 6, 1953.

[1] These investigations were supported by the Atomic Energy Commission, Contract No. AT(45-1)-247.

[2] Present address: Radioisotope Unit, Veterans Administration, Medical Teaching Group Hospital, Memphis, Tenn.

[3] Obtained from Abbott Laboratories, North Chicago, Illinois.

[4] The crystalline zinc insulin, 26 units/mg. was a gift of the Eli Lilly Company.

product was hypoglycemic to rabbits, assayed according to prediction by the *in vitro* method of Groen, *et al.* (1952) and was shown chromatographically to contain only protein bound radioactivity. The activity of the product was of the order of 300 μc/mg and the dose ranged from 0.158 to 1.76 units. In certain control experiments iodinated human serum albumin labeled with I^{131} (IHSA)[3] was administered in doses of 1 μc., containing about 20 μgm. of protein. Carrier-free radioactive iodine was obtained from Oak Ridge.

2. *Experimental Procedure*

Female Sprague-Dawley rats, raised in the local colony and weighing from 160 to 278 grams, were used in all experiments and were fed the stock diet *ad libitum*. All isotopic preparations were injected in the tail vein in 0.25 ml. of 0.9% NaCl using ether anesthesia. After allotted time intervals, the animals were reanesthetized and exsanguinated. The liver was immediately removed and rinsed in ice cold alkaline KCl. In some experiments the liver was first perfused *in situ* with an ice cold modified Ringer's solution (Gey and Gey, 1936). The perfusion medium was introduced through a 20 gauge needle from a 30 ml. syringe. The needle was inserted in the portal vein and tied in place, the superior vena cava was cut open, and the liquid was forced through the liver under gentle pressure. This organ was usually completely blanched after 1–3 ml. were administered, but the procedure was always continued until the entire 30 ml. were used. The entire operation did not take over two minutes.

All subsequent work was carried out in the cold room or under similar conditions of refrigeration. A 10% homogenate was prepared using isotonic sucrose solution and an aliquot was taken as representative of the intact liver. Nuclear, mitochondrial, microsomal and residual fractions were obtained by successive 10 minute centrifugations of the homogenate at 600 $\times$g., 11,000 $\times$g. and 100,000 $\times$g., respectively, the residual fraction being defined as the supernate from the 100,000 $\times$g. spinning. The radioactivity of these fractions was directly estimated with a cup-type gamma counter and the results expressed as per cent administered radioactivity/gram of liver or as per cent distribution among the liver intracellular structures.

RESULTS

I. *Distribution Studies*

The following questions were considered in the investigation of the problems of the cellular penetration and intracellular fixation of labeled insulin and thyroxine: (1) Does the presence of radioactivity in the liver indicate that the hormone has penetrated the cytoplasm? Perfusion studies were executed to show whether radioactivity is easily removable or not and thus give an initial answer. More definitive would be the finding of strong and characteristic association of radioactivity with various subcellular fractions, especially the particulate elements (nuclei, mitochondria and microsomes). (2) Can the association of radioactivity with the subcellular fractions be attributed to passive phenomena occurring subsequent to homogenization of the liver, or can such information be shown to be dependent on cellular integrity, thus indicating the active and physiological nature of the binding processes? This question was directly investigated by comparison of distribution data following intra-

venous administration of the labeled factor with similar data obtained by mixing the labeled factor with an homogenate. (3) Does the fact that insulin I^{131} is a foreign protein entirely account for the data obtained? This question was investigated by executing similar studies employing IHSA. (4) What role does catabolism and subsequent distribution of radioactive iodide play in characterizing hormone distribution?

A. *Insulin-I^{131}*. Inspection of Table 1 shows that within 5 minutes after the intravenous administration of insulin-I^{131}, the concentration within the liver was 2.10% of the administered dose/gram of tissue. This value clearly indicates an active removal of insulin from the blood by the liver. Furthermore, the major portion of the radioactivity in the liver was associated with the non-nuclear fraction of the homogenate and was distributed approximately equally among the various subcellular constituents within

TABLE 1. THE LOCALIZATION OF RADIOACTIVITY IN RAT LIVER FIVE MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF INSULIN-I^{131}

	In vivo		*In vitro*
	Unperfused (I)	Perfused (II)	(III)
% of dose/gram of liver	2.10 ± 0.19*	1.94 ± 0.18	
Intracellular distribution of radioactivity in liver, %			
Nuclear Fraction	10.8 ± 0.42	8.4 ± 0.38	8.40†
Motochondrial Fraction	26.8 ± 1.58	27.0 ± 2.68	6.20
Microsomal Fraction	32.3 ± 0.79	34.0 ± 1.69	14.0
Residual Fraction	33.8 ± 1.23	30.9 ± 3.08	72.0
Recovery	104 ± 1.28	100 ± 1.32	101
Number of experiments	5	9	2
Dose, units of insulin	0.295–1.57	0.158–1.76	0.811

* Mean ± standard error.
† No statistical treatment is given when less than 4 experiments are reported.

this compartment. Perfusion showed this radioactivity to be firmly bound since only a statistically insignificant portion could be removed and the intracellular distribution remained unaffected. This finding is in accord with the demonstration of the rapid and firm binding of insulin to tissue *in vitro* (Stadie, *et al.* 1949, Hills and Stadie, 1952, and Haugaard and Marsh, 1952).

It might be argued that insulin-I^{131} is confined to the cell wall and/or is in solution within the cell. Consequently, the distribution data presented in these studies would be characteristic of a passive phenomenon such as adsorption occurring subsequent to homogenization. If this were so, then addition of insulin-I^{131} to an homogenate should result into its distribution being very similar to that found for the *in vivo* experiment. Comparison of columns I and III of Table 1 shows this not to be the case; it therefore

follows that insulin-I^{131} was taken into the cell and distributed among the various subcellular components by active processes characteristic of cellular integrity.

B. *Thyroxine-I^{131}*. Within 5 minutes after the intravenous administration of L-thyroxine-I^{131} the liver contained 8.35% of the administered dose per gram (Table 2). However, binding of the hormone was not complete since approximately 40% of the radioactivity could be removed by perfusion. Furthermore, perfusion losses were widely different for the various subcellular constituents, thus resulting in a marked difference in distribution as compared with the unperfused liver. Analysis of the data for columns I and II shows the following changes had occurred: nuclear fraction, +22%; mitochondrial fraction, −51%; microsomal fraction, −60%; residual fraction, −57%.

TABLE 2. THE LOCALIZATION OF RADIOACTIVITY IN RAT LIVER FIVE MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF THYROXINE-I^{131}

	In vivo		*In vitro*
	Unperfused (I)	Perfused (II)	(III)
% of dose/gram of liver	8.35 ±0.59	5.10 ±0.31	
Intracellular distribution of radioactivity, %			
Nuclear Fraction	20.2 ±0.51	40.3 ±0.94	17.2
Mitochondrial Fraction	48.8 ±1.88	39.6 ±1.32	12.0
Microsomal Fraction	23.9 ±2.69	15.4 ±1.10	40.7
Residual Fraction	6.10 ±0.91	4.16 ±0.44	30.2
Recovery	98.6 ±1.96	99.5 ±2.06	100
Number of experiments	4	4	2
Dose, μgm. L-thyroxine	0.09	0.09	0.09

Comparison of columns I and III of Table 2 leads to the same general conclusion as stated for insulin-I^{131}; namely, L-thyroxine-I^{131} was taken into the liver cell and distributed among the subcellular constituents by active processes characteristic of cellular integrity. However, since an appreciable fraction of the localized radioactivity could be removed by perfusion, and the intracellular distribution be affected in both absolute and relative terms, it is obvious that thyroxine binding was neither as firm nor as straightforward as in the case of insulin-I^{131}. This property will be discussed in one of the following sections of this report.

C. *Iodinated Human Serum Albumin-I^{131} (IHSA)*. Table 3 presents the data from experiments in which the intracellular distribution of IHSA was studied. These data are in distinct contrast to those obtained for insulin-I^{131} (Table 1). Within 5 minutes after intravenous administration the liver contained only 0.81% of the dose/gram of tissue and approxi-

TABLE 3. THE LOCALIZATION OF RADIOACTIVITY IN RAT LIVER FIVE MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF IHSA

	In vivo		*In vitro*
	Unperfused (I)	Perfused (II)	(III)
% dose/gram of liver	0.81 ±0.06	0.31 ±0.01	
Intracellular distribution of radioactivity, %			
Nuclear Fraction	6.66 ±0.41	7.97 ±1.01	3.68
Mitochondrial Fraction	7.36 ±0.63	19.7 ±0.83	2.04
Microsomal Fraction	10.4 ±1.24	29.3 ±0.31	1.62
Residual Fraction	74.0 ±3.75	45.5 ±1.61	92.8
Recovery	98.0 ±3.11	102 ±3.10	100
Number of experiments	4	4	2
Dose, μgm. protein	20	20	10

mately 62% of that was removable by perfusion. Although perfusion resulted in a different distribution among the subcellular components, it should be pointed out that the main source of the perfusible radioactivity was in the residual fraction. This fraction, by reason of the method of its isolation, represents not only the non-particulate portion of the cell but also the intra- and extracellular fluids. Therefore, the radioactive material found in this fraction must be considered as unfixed to any subcellular structural element, and probably for that reason easily removable by perfusion. Comparison of column I with column II and I with III is in accord with this analysis. The similarity in distribution, the finding of the major portion of the radioactivity in the residual fraction for all experimental groups, supports the argument that, of the IHSA present in the liver, most of it is unfixed to cytostructural elements and is either in solution extracellularly and/or in solution intracellularly and in rapid equilibrium with the extracellular fluid.

The values for the mitochondrial and microsomal fractions indicate perfusion caused relatively little loss of radioactivity from these structures. The significance of this is conjectural at present but it may be offered as unproved assumption that this firm binding may be related to the antigenicity of the IHSA.

D. *Iodide-I^{131}*. In view of the preceding sections the data of Table 4 can be analyzed quite readily. After the intravenous administration of iodide-I^{131} almost all of the radioactivity in the liver was in solution intracellularly and/or extracellularly. Although perfusion resulted in a 63% loss, the similarity in distribution data for columns I and II shows that the radioactivity associated with the nuclear, mitochondrial and microsomal elements was in rapid equilibrium with the radioactivity in solution. Furthermore, that I^{131} which was found associated with cytostructural elements

TABLE 4. THE LOCALIZATION OF RADIOACTIVITY IN RAT LIVER FIVE MINUTES AFTER THE INTRAVENOUS ADMINISTRATION OF IODIDE-I^{131}

	In vivo		*In vitro*
	Unperfused (I)	Perfused (II)	(III)
% of dose/gram of liver	0.49 ± 0.03	0.18 ± 0.02	
Intracellular distribution of radioactivity, %			
Nuclear Fraction	6.90 ± 0.28	5.87 ± 0.96	6.76
Mitochondrial Fraction	5.55 ± 1.20	5.90 ± 1.42	4.79
Microsomal Fraction	4.94 ± 0.26	6.26 ± 0.55	3.33
Residual Fraction	89.0 ± 4.82	82.8 ± 7.43	81.2
Recovery	107 ± 6.34	101 ± 5.58	96.1
Number of experiments	4	4	2
Dose	Carrier-free	Carrier-free	Carrier-free

could be obtained merely by mixing the isotopic material with an homogenate; consequently, the distribution values appear to be characteristic of passive phenomena, probably adsorption, and not of the same mechanisms operative in the studies with insulin-I^{131} and thyroxine-^{131} *in vivo*.

II. *Time Studies*

After the intravenous administration of either insulin-I^{131} or thyroxine-I^{131}, a semi-logarithmic plot of the per cent of the administered dose/gram of liver with respect to time shows the loss of radioactivity to correspond to two straight line phases (Fig. 1). These plots are individually characteristic for the hormone considered and are distinctly different with respect to comparable phases. That the earlier phase, 0–30 minutes, is related to mixing of the hormone in the vascular and extracellular fluids and to translocation across cell membranes is undoubtedly of importance in interpretation. However, the distribution data presented below clearly shows these phases to be representative of underlying intracellular phenomena characteristic for the hormone.

A. *Insulin-I^{131}*. Within 5 minutes after intravenous administration of insulin-I^{131} the liver contained 2.10% of the administered radioactivity /gram (Table 5). At 30 minutes this value had fallen to 0.69% and at 60 minutes to 0.51%. These losses amounted to 68% and 76%, respectively. Further inspection of Table 5 reveals that radioactivity was lost from all fractions as the duration of the experiment continued but that these losses appeared to occur at rates which were individually different for each fraction.

This conclusion becomes manifest by inspection of Figure 2 which shows the above mentioned differential loss rates to be reflected in sharp changes in intracellular distribution. When the liver content of insulin-I^{131} was

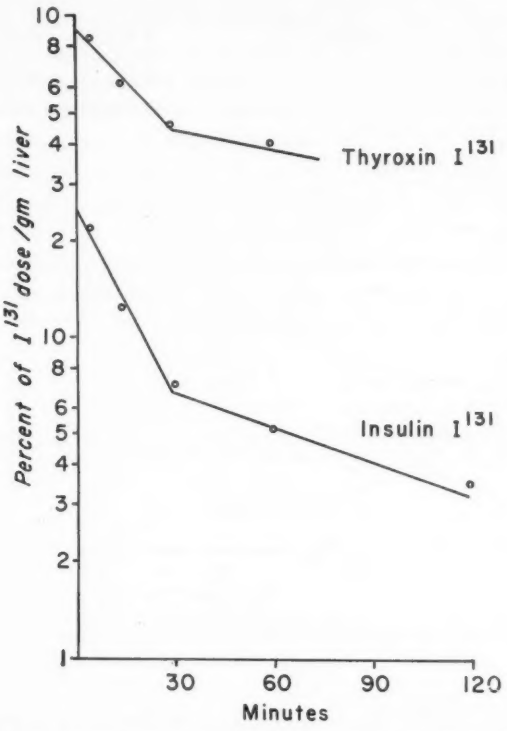


FIG. 1. The relation between time and the concentration of radioactivity in rat liver after intravenous administration of thyroxine-I^{131} and insulin-I^{131}.

2.10% of the administered dose/gram, the distribution of intracellular radioactivity fell into two main groups, the microsomal and residual fractions having more than the nuclear and mitochondrial fractions. By the time the tissue concentration of isotope had fallen 68% (30 minutes) the residual fraction contained the greatest portions of the radioactivity present, the nuclear and mitochondrial fractions were intermediate in value and the microsomal fraction contained least. This pattern of distribution persisted for the remainder of the interval under study, the only changes noted being quantitative rather than qualitative. These curves may be described as showing that, relative to the rate of loss of radioactivity of liver tissue as a whole the microsomal rate of loss was faster, that for the nuclear and mitochondrial fractions the same as for the whole tissue, and that for the residual fraction being slower than that for the whole liver. These conditions prevailed until a condition (30–60 minutes) was obtained under which the relative distribution within the cell remained constant.

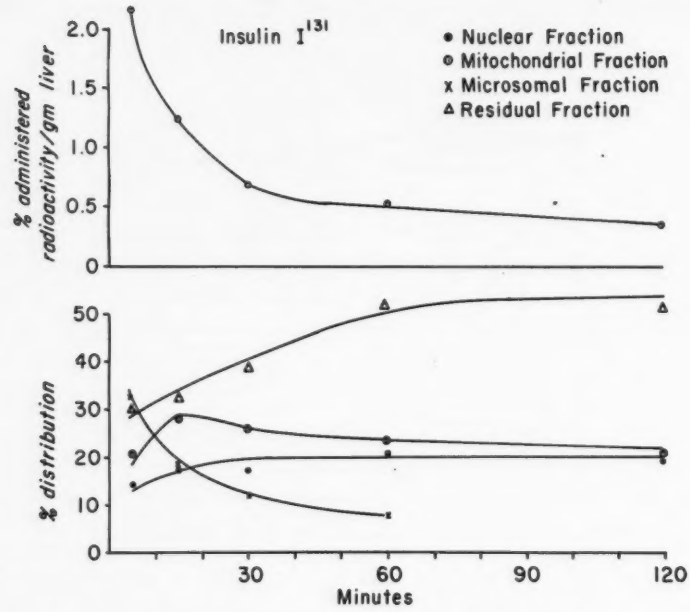


FIG. 2. Relation between time and distribution of radioactivity in rat liver after intravenous administration of insulin-I^{131}.

B. *Thyroxine-I^{131}*. Within 5 minutes after the intravenous administration of L-thyroxine-I^{131} the liver contained 8.35% of the administered dose/gram (Table 5). This value fell to 4.50% at 30 minutes and 3.88%

TABLE 5. THE DISTRIBUTION OF RADIOACTIVITY IN RAT LIVER AT VARIOUS TIME INTERVALS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF INSULIN-I^{131} OR THYROXINE-I^{131}*

Time interval, min.	Insulin-I^{131}				Thyroxine-I^{131}			
	5	15	30	60	5	15	30	60
Liver	2.10± 0.19	1.23± 0.05	0.69± 0.01	0.51± 0.04	8.35± 0.59	6.20± 0.44	4.50± 0.09	3.88± 0.12
Nuclear Fraction	0.23± 0.01	0.21± 0.02	0.12± 0.01	0.11± 0.02	1.68± 0.04	0.97± 0.03	0.97± 0.07	0.77± 0.09
Mitochondrial Fraction	0.56± 0.03	0.34± 0.01	0.18± 0.01	0.12± 0.01	4.06± 0.16	1.33± 0.05	1.11± 0.04	1.02± 0.05
Microsomal Fraction	0.68± 0.02	0.22± 0.01	0.08± 0.006	0.04± 0.005	1.99± 0.22	2.54± 0.03	1.70± 0.04	1.48± 0.10
Residual Fraction	0.71± 0.03	0.41± 0.03	0.27± 0.01	0.26± 0.01	0.51± 0.08	1.19± 0.07	0.86± 0.04	0.66± 0.02
Recovery	104± 1.28	95.9± 2.35	94.2± 2.41	104± 5.91	98.6± 1.96	97.3± 1.59	103± 2.56	101± 0.92
Dose	0.245 units	0.245 units	0.245 units	0.245 units	0.09 /μgm.	0.09 /μgm.	6.0 /μgm.	0.09 /μgm.

* Data are expressed as % of administered dose/gram of liver or/total fraction from 1 gram of liver.

at 60 minutes, those losses amounting to 46% and 53%, respectively. Coincident with this picture was a series of striking changes in the intracellular distribution of radioactivity. The amount of I^{131} associated with the nuclear and mitochondrial fractions either diminished during the entire period under study or was held constant for a brief interval. On the other hand, there was an absolute as well as relative gain in the amount of radioactivity associated with the microsomal and residual fractions within 15 minutes after the experiment began. These increments amounted to 33% and 143%, respectively. Once this initial phase had occurred, the relative distribution remained reasonably constant over the remainder of the experimental period (Fig. 3). This constancy of pattern, as well as

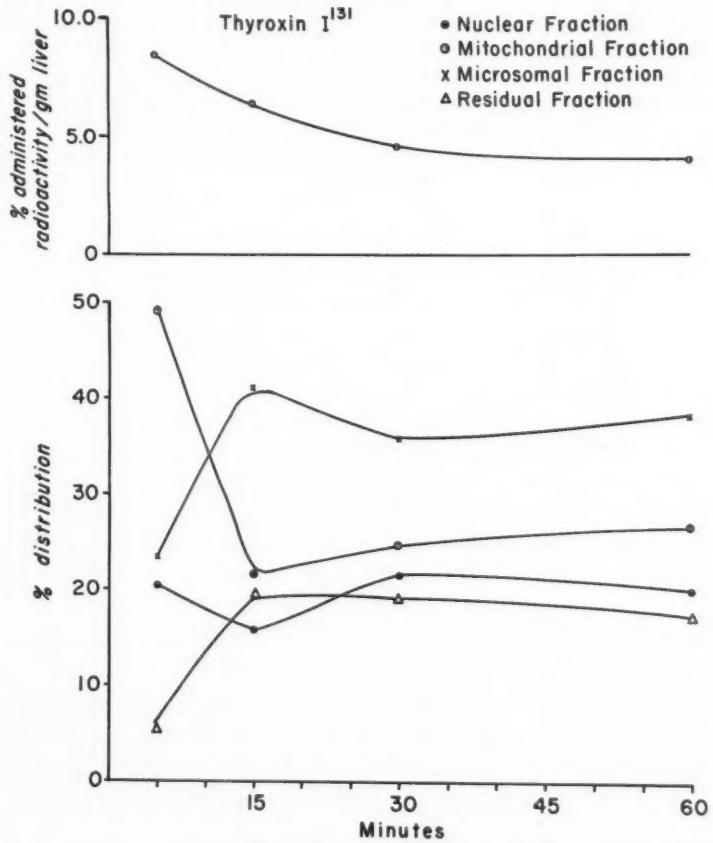


FIG. 3. Relation between time and distribution of radioactivity in rat liver after intravenous administration of thyroxine-I^{131}.

the quantitative data, is in good agreement with the findings of Lipner, *et al.* (1952a) in which the time interval employed ranged from 3 through 24 hours.

C. *Iodinated Human Serum Albumin-I^{131} (IHSA).* The relations observed following the intravenous administration of IHSA (Fig. 4) are roughly similar to those observed for insulin-I^{131} (Fig. 2). The most signifi-

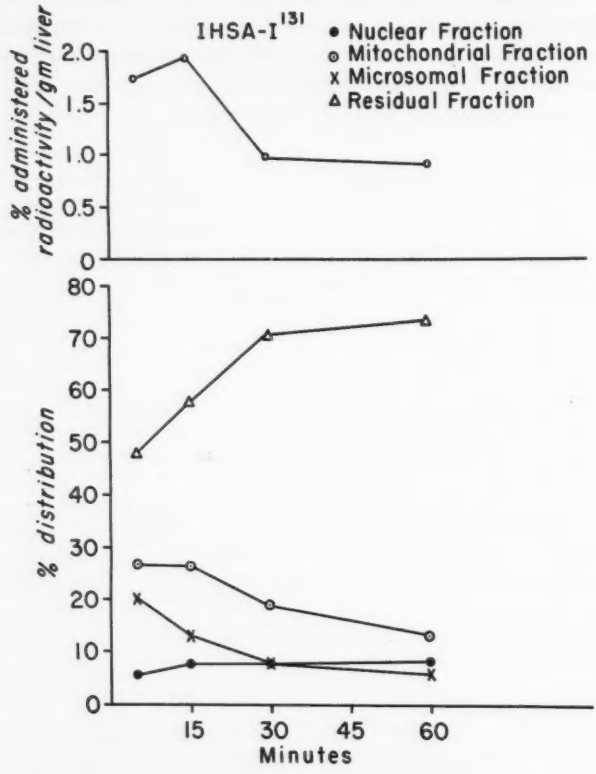


FIG. 4. Relation between time and distribution of radioactivity in rat liver after intravenous administration of IHSA.

cant difference is that for IHSA the role of the residual fraction was much more pronounced. It should be further noted that by 30 minutes the persistent distribution relationships had appeared with the major portion of the radioactivity being associated with the residual fraction.

III. *Thyroxine-I^{131} Binding to Cytostructural Elements*

It was previously shown that an insignificant portion of the insulin radioactivity could be removed by perfusion (Table 1) whereas 39% of the

isotope could be washed out after thyroxine-I^{131} administration (Table 2). Perfusion also resulted in distinctive changes in the intracellular distribution of radioactivity for the thyroxine experiment, whereas none could be observed for the insulin experiment. Finally, after the intravenous administration of thyroxine-I^{131}, both the absolute and relative amounts of liver radioactivity that could be removed by perfusion diminished as the duration of the experiment continued (Table 6). The amount of radioactivity present/gram of liver at 60 minutes was 45% of the 5 minute value, whereas the amount that could be removed by perfusion was only 6.8%.

These results may be explained by the following alternatives: (1)

TABLE 6. EFFECT OF PERFUSION AT DIFFERENT TIMES AFTER INTRAVENOUS ADMINISTRATION OF THYROXINE-I^{131}

	5 min.	15 min.	60 min.
% Administered radioactivity/gm. liver			
1) Before perfusion	8.35 ± 0.59	6.20	3.88
2) After perfusion	5.10 ± 0.31	5.04	3.66
% Administered radioactivity removed	3.25	11.6	0.22
% Localized radioactivity removed	38.7	18.7	5.67
Dose, L-thyroxine/μgm.	0.09	0.09	0.09

Thyroxine-I^{131} is entirely bound in the liver in two compartments, being easily removed from one by perfusion and not removed from the other. (2) Thyroxine-I^{131} is *not* entirely bound in the liver, one fraction being soluble in the extracellular fluid or in rapid equilibrium with it and easily removed by perfusion and the other not removed by perfusion.

If only thyroxine in solution is removable from these fractions by dialysis then a critical test of the above alternatives could be effected by the use of this technique. If the first alternative is correct, dialysis of the unfractionated homogenate and the residual fraction should result in little or no loss of radioactivity from *both*. If the second is correct, marked losses should appear in both. The dialysis consisted of 3 to 4 changes of M/15 phosphate buffer, pH 7.4, lasting over 18 hours and with constant agitation. The results are presented in Table 7.

Inspection of the values for the liver and the residual fraction for the 15 minute series shows no appreciable loss of label from the former and little from the latter after dialysis. At 60 minutes, the loss from the residual fraction approximates that for the perfusion series. However, this is most probably the result of the removal of radioactive iodide arising from thyroxine-I^{131} catabolism. This argument is supported by the fact that, under identical conditions, no I^{131} can be found in the thyroid after thyroxine-^{131} administration until 60 minutes has elapsed (Elgee *et al.*, unpublished data). Consequently these results support the alternative that thyroxine is entirely bound in the liver in two compartments and

TABLE 7. EFFECT OF PERFUSION AND DIALYSIS ON THE BINDING OF RADIOACTIVITY AT DIFFERENT TIMES AFTER INTRAVENOUS ADMINISTRATION OF THYROXINE-I^{131}

% Localized radioactivity removed by:	15 min.		60 min.	
	Perfusion	Dialysis	Perfusion	Dialysis
Liver	18.6	2.1	5.7	1.9
Nuclear Fraction	+53 (gain)	8.2	+9 (gain)	16.5
Mitochondrial Fraction	10.7	6.5	20.7	16.2
Microsomal Fraction	32.8	0.6	5.7	4.7
Residual Fraction	46.8	8.4	22.6	16.3
Number of experiments	4	2	4	2
Dose, L-thyroxine, μgm.	0.09	0.09	0.09	0.09

these compartments may be operationally defined in terms of perfusion and dialysis experiments.

The results of perfusion or dialysis at 15 or 60 minutes after thyroxine administration exhibit the following: (1) Dialysis showed that, with the passage of time, thyroxine-I^{131} binding was less firm. This is particularly evident with respect to the nuclear and mitochondrial fractions. (2) Perfusion showed that thyroxine-I^{131} was more firmly bound at 60 minutes than at 15 minutes. In addition, distribution values showed thyroxine binding was, with respect to time, less labile for the microsomal and residual fractions and more labile for the mitochondrial fraction. The values for the nuclear fraction are curious inasmuch as they are the result of a net *gain* in radioactivity following perfusion.

In view of these results the predicted concomitants of perfusion studies were observed, thus supporting the view that insulin is firmly bound whereas thyroxine binding is specified in terms of time and locus. Further evidence of the firmness of thyroxine-I^{131} binding is provided by the fact that the protein of the various subcellular fractions may be denatured and precipitated and still retain radioactivity (Lipner *et al.*, 1952a).

DISCUSSION

The following appears to be the course of events when insulin-I^{131} or thyroxine-I^{131} is presented to the liver by the blood. The hormone is actively concentrated by the liver. Five minutes after the administration, the liver:blood ratio for thyroxine-I^{131} was approximately 8:1 and for insulin-I^{131} about 4:1. These ratios are low since the liver, free of a low activity blood contaminant, would, per gram, have more isotope. Concentration, necessitating cellular integrity, appears to involve cell wall penetration, entry into the cytoplasm and fixation to the nucleus and the different cytoplasmic fractions.

Upon entry into the cytoplasm the immediate distribution and fixation of the hormone is different from that occurring after a short time interval. It would appear that fixation is a complex progressive phenomenon ultimately resulting in the establishment of a stable set of relationships. This stable state appears within 30 to 60 minutes after hormone administration.

The above description is applicable to both insulin-I^{131} and thyroxine-I^{131}. The latter case, however, may be viewed with a good deal more certainty in view of its distinct contrast to the insulin results and because of the nature of the perfusion and binding studies. Such studies show distinctive time and locus characteristics and strongly support the argument of the preceding section.

Viewed in its entirety these data indicate a general description of the role of the liver in the metabolism of certain hormones and show that the details of this description are individually specified by the hormone under consideration. An *in vitro* metabolic action of thyroxine has been demonstrated for nuclei and mitochondria with respect to glutamate oxidation (Feldott and Lardy, 1951) and for mitochondrial oxidative phosphorylation (Martius and Hess, 1951). Stadie and co-workers (1949) have shown that the first step in the insulin stimulation of glucose utilization by rat diaphragm is the firm and concentrative binding of the hormone. Whether the description offered in the present report is concerned with the mode of action of these hormones or the manner in which they are metabolized by the liver cannot be stated at the present. It would seem, however, that this question is pointless, in reference to the level of metabolic organization considered here. Indeed, this problem revolves about the question of the identity of the phenomena of hormone action and hormone breakdown.

Regardless of the above points, however, these data do elucidate the fate of certain hormones at the cellular level. The reports of Lipner *et al.* (1952a, 1952b) deal with some of the questions under present study. Comparisons cannot be made since the conditions of the experiment, physiological state of the animals, treatment of the liver and hormone dosage were widely different from those employed here. However, these workers did show that thyroxine-I^{131} distribution in the *in vitro* experiment was no different from the *in vivo* experiment. In the face of this, their argument that thyroxine may, and does, penetrate the cell is not understandable in terms of their own data. Similar experiments presented here (Table 1) are totally different and are in harmony with the conclusion that thyroxine penetrates the intracellular milieu and that this process is an active one.

Levine and co-workers (1950) have shown that the volume of distribution of galactose corresponds to the vascular and extracellular fluids and that after insulin administration this volume expands corresponding to that for the total body water. On the basis of this finding they suggest that the action of insulin is on the control of cell membrane permeability. This hypothesis has found support in the investigations of Wick *et al.* (1951). The data presented in the present report show that within 5 minutes after administration *at least* 66% of the insulin in the liver is intracellular and bound to cytostructural elements; within 1 hour this value only falls to 49%. Furthermore, these data are obtained despite an 11-fold dosage range. In view of this, it would seem unreasonable to consider the cell wall as the sole site of insulin action. What the intracellular role is cannot be considered at present since there is no information concerning the metabolic effects of insulin in terms of the various subcellular components.

Two points of methodological importance arise here. Firstly, the time interval observed was 60 to 120 minutes. This limitation arose from the following considerations: 1) The present investigation was concerned with questions about the *immediate fate* of the labeled factors, whether they penetrated the cytoplasm and, if so, became fixed to the various cytostructural elements in a definitive manner. The answers to these questions would have been obscured had longer term studies been executed since the labeled products of hormone catabolism would have become increasingly predominant. In view of this, the most definitive answers to the above questions are obtained with the 5 minute determinations. 2) Disregarding the previous argument, a short time interval was necessary for technical reasons. The continuous catabolism of the labeled hormone and the dilution by endogenous hormone results in radioassay values of questionable significance. This problem might be avoided if a more highly labeled preparation were available. Unfortunately, this is not the case.

Secondly, the question of dosage effect is of some importance. The data for Table 1 was compiled from pooled values of experiments involving doses ranging from 0.158 units of insulin to 1.76 units/200 gram rat, a range greater than 11 fold. Inspection of the variation of the resultant data (standard error) shows no such range; furthermore, analysis of the raw values showed no significant variations. The data for the insulin time studies was obtained with doses of 0.245 units/200 gram rat. As for the thyroxine studies, with the exception of the 30 minute determination for the time curves, all experiments employed only 0.09 μgm./200 gram rat. Consequently, it is reasonable to assume that hormone dosage was of no consideration with respect to the type of determination presented in this paper.

SUMMARY

Using I^{131} as a label, it was shown that the intravenous administration of either thyroxine or insulin to the rat is followed by concentration of these hormones in the liver. This concentration process involves passage through the cell wall and fixation to the various cytoplasmic structures and to the nucleus. The distribution of thyroxine-I^{131} among the cytostructural elements is different from that for insulin-I^{131} and such differences are maintained with respect to the time studies.

Insulin, localized in the rat liver, appears to be completely resistant to removal by perfusion whereas this technique removes a relatively large quantity of the localized thyroxine.

The nature of the binding of thyroxine intracellularly changes with respect to time and is a characteristic of the cytostructural element under consideration.

REFERENCES

ELGEE, N. J., N. D. LEE AND R. H. WILLIAMS: Unpublished data.

FELDOTT, G. AND LARDY, H. A. *Federation Proc.* **10:** 182. 1951.

FERREBEE, S. W., B. B. JOHNSON, J. C. MITHOEFER AND J. W. GARDELLA: *Endocrinology* **48:** 277. 1951.

GEY, G. O. AND M. K. GEY: *Am. J. Cancer* **27:** 45. 1936.

GROEN, J., C. E. KAMMINGA, A. F. WILLEBRANDS AND J. R. BLICKMAN: *J. Clin. Investigation* **31:** 97. 1952.

HAUGAARD, N. AND J. B. MARSH: *J. Biol. Chem.* **194:** 33. 1952.

HILLS, A. G. AND W. C. STADIE: *J. Biol. Chem.* **194:** 25. 1952.

LEVINE, R., M. S. GOLDSTEIN, E. HUDDLESTON AND S. P. KLEIN. *Am. J. Physiol.* **163:** 70. 1950.

LIPNER, J. H., S. B. BARKER AND T. WINNICK. *Endocrinology* **51:** 406. 1952a.

LIPNER, J. H., H. M. KLITGAARD, S. B. BARKER AND T. WINNICK: *Federation Proc.* **11:** 96. 1952b.

MARTIUS, C. AND HESS B. *Arch. Biochem. Biophys.* **33:** 486. 1951.

STADIE, W. C., N. HAUGAARD, J. B. MARSH AND A. G. HILLS: *Am. J. Med. Sc.* **218:** 265. 1949.

WICK, A. N., D. R. DRURY, E. M. MACKAY: *Ann. N. Y. Acad. Sci.* **54:** 684. 1951.

SENSITIVITY TO INSULIN DURING ADRENOCORTICAL RESPONSE TO COLD STRESS IN RATS[1]

TAG E. MANSOUR[2] AND WILLIAM F. HEWITT, JR.[3]

Department of Physiology, Howard University School of Medicine, Washington, D. C.

IN 1946 Selye described cold as an alarming stimulus capable of producing all of the systemic effects characterizing the general adaptation syndrome. He had reported changes in carbohydrate metabolism in rats exposed to cold: a marked hyperglycemia immediately following initial exposure, hypoglycemia on the second day of cold, and hyperglycemia again beginning on the third day and lasting two weeks (stage of resistance).

Many alarming stimuli including cold cause increased adrenocortical activity characterized by enlargement of the gland and reduction of its content in ascorbic acid and cholesterol (Selye, 1937; Long, 1947; and Sayers and Sayers, 1948). Masson (1941) found that the hypoglycemia-producing effect of insulin (that is, insulin sensitivity) is increased considerably in the stage of resistance during chronic exposure to a cold environment. More recently Cheng and Sayers (1949) produced hypersensitivity to insulin by administering desoxycorticosterone acetate to adrenal-demedullated rats. In view of these reports, it appeared to be of interest to measure simultaneous changes in adrenocortical activity and sensitivity to insulin during chronic stress, since information on these points was lacking.

METHODS

Adult (150 to 250 gm.) rats from a single colony were used. Sugar was estimated by the orcinol reaction (Vasseur, 1948) on 0.5 cc. samples of tail blood. Bleeding was improved by infiltration of the base of the tail with 1% solution of '*Intracaine*' (Squibb) and by immersion in water at about 45° C. Rats were fasted for 24 hours, a blood sample was taken, 1¼ U of insulin per kg. of body weight were injected subcutaneously, and a second blood sample was taken 30 minutes later. Absolute and per cent reduction of blood sugar was used as an index of sensitivity to insulin.

Cold stress consisted of placing rats, two to a cage, in a room kept at about 5° C. for 1 day, 6 days or 9 days continuously. After 24 hours' fast at the end of the stress period, the animals were removed from the cold room and killed by a blow on the head after

Received for publication May 11, 1953.

[1] Aided by a grant from Merck & Co.

[2] Present address: Department of Pharmacology, Western Reserve University School of Medicine, Cleveland, Ohio.

[3] Present address: Department of Physiology and Pharmacology, Des Moines Still College of Osteopathy and Surgery, Des Moines, Iowa.

insulin sensitivity had been determined again. The adrenal glands were removed, dissected free of fat and connective tissue, and weighed to the nearest 0.1 mg. on a Roller-Smith microtorsion balance. Ascorbic acid was estimated in the right adrenal by the method of Roe and Kuether (1943) as modified by Sayers, Sayers and Woodbury (1948). Cholesterol was measured in the left adrenal by the method of Bloor, Pelkan and Allen (1922) after grinding with sand and extraction with 3:1 alcohol-ether solution.

Insulin sensitivities for all stressed rats were measured three days before, and within three hours after, exposure to cold. Insulin sensitivity was determined in several additional control animals which were not stressed, and after 9 days' cold in two rats whose pre-stress sensitivity had not been measured. These two animals replaced rats in which it was impossible to obtain post-stress blood samples.

RESULTS

Blood sugar values are summarized in Table 1 and Figure 1. Rats exposed to cold for 1 day showed no significant change in insulin sensitivity. However, there was a slight rise of the blood sugar. Those exposed for 6 or 9 days, on the other hand, exhibited statistically significant hyperglycemia

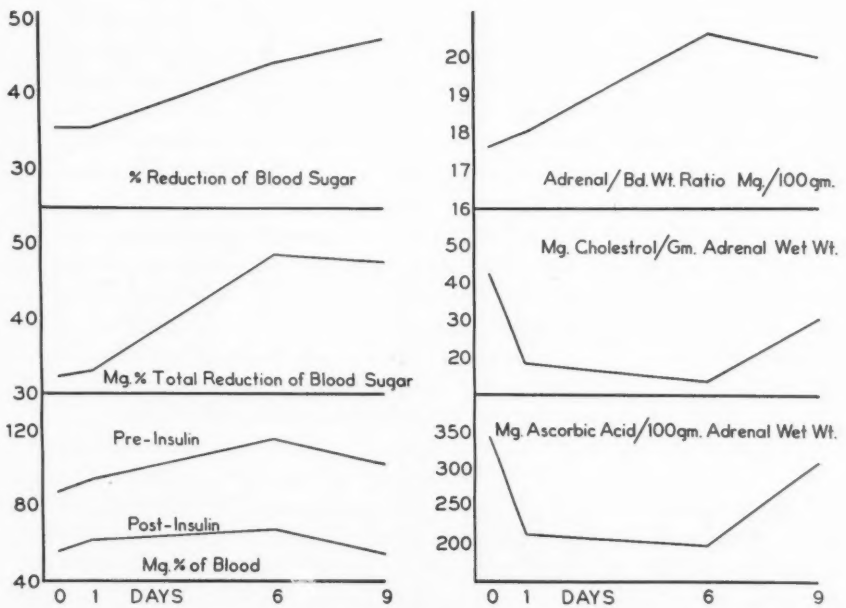


FIG. 1. Graphic representation of the effect of exposure to cold stress for a certain time (abscissa) on: Left: Blood sugar and sensitivity to insulin; Right: Adrenal body weight ratio and cholesterol and ascorbic acid content of the adrenal glands.

TABLE 1. THE EFFECT OF COLD STRESS ON INSULIN SENSITIVITY

Group	Treatment	No. of rats	Sugar, mg. per 100 cc. of blood				Decrease in blood sugar			
			Pre-insulin		Post-insulin		Mg. per 100 cc.		Per cent	
			Mean S.E.†	P*	Mean S.E.	P	Mean S.E.	P	Mean S.E.	P
I	Control	40	87±2.7		55.0±1.8		32 ±2.2		35.5±1.8	
II	a. Pre-stress	10	96±3.0		61.5±3.7		34.5±3.7		35 ±3.7	
	b. 24 hrs. stress		94±5.1	I–IIb <0.05	60.5±3.5		33 ±3.2		35.5±2.4	
III	a. Pre-stress	10	79±3.8		55.0±4.0		24 ±2.2		30.5±2.5	
	b. 6 days stress		116±3.2	a–b <0.01 I–IIIb <0.01	67.5±4.6	a–b =0.05 I–IIIb <0.01	48.5±3.9	a–b <0.01 I–IIIb <0.01	44 ±3.4	a–b <0.01 I–IIIb <0.05
IV	a. Pre-stress	11	81±5.3		51.0±4.0		29 ±3.5		36 ±3.2	
	b. 9 days stress		102±4.2	a–b <0.01 I–IVb <0.01	54.0±5.3		47.5±3.0	a–b <0.01 I–IVb <0.01	47 ±3.1	a–b <0.05 I–IVb <0.01

* Probability of a chance occurrence of the difference between the means from Fisher's table of t values (Fisher 1950). The confidence level is given for all differences found to be statistically significant.

† Standard error of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

TABLE 2. THE EFFECT OF COLD STRESS ON THE ADRENAL CORTEX

Group	Treatment	No. of rats	Ascorbic acid		No. of rats	Cholesterol		No. of rats	Adrenal/body wt.	
			Mg./100 gms.	P		Mg./gm.	P		Mg./100 gm.	P
I	None	22	344±20.5		16	42.9±2.3		22	17.6±0.5	
II	24 hours stress	10	213±13.1	I–II <0.01	8	18.0±1.7	I–II <0.01	10	18.0±0.6	
III	6 days stress	12	196±11.4	I–III <0.01	11	13.9±1.6	I–III <0.01	12	20.6±1.2	I–III =0.02
IV	9 days stress	10	309±8.7		12	30.6±2.7	I–IV <0.01	10	20.0±1.1	I–IV <0.05

as noted by Selye (1938), and increased sensitivity to insulin. The apparent increase in insulin sensitivity from 6 to 9 days is not statistically significant.

Adrenal ascorbic acid, cholesterol, and relative weight values are summarized in Table 2 and Figure 1. Pre-stress ascorbic acid and cholesterol observations agree with those of Shepherd and others (1952). Cold stress for 24 hours provoked a marked and significant fall in adrenal ascorbic acid and cholesterol, without effect upon the gland's relative weight, and these decreases were maintained at 6 days of cold. Relative adrenal weight was elevated significantly at 6 and at 9 days of stress. By 9 days, the difference between stress and pre-stress ascorbic acid values had disappeared, the apparent difference being outside statistical confidence limits, but adrenal cholesterol was still depressed below control measurements.

It should be noted here that although the adreno-cortical activity was high at the one day period the insulin sensitivity was not significantly changed. At the 9th day, furthermore, insulin sensitivity and the blood sugar level were still high while adreno-cortical activity was at or approaching normal levels.

DISCUSSION

The results of this study clearly indicate that the correlation between changes in blood sugar and insulin sensitivity on the one hand, and corticoadrenal activity on the other hand, was not constant. For example, on the first day the cortical activity was markedly high. This was indicated by the marked depletion of both cholesterol and ascorbic acid content of the gland and an increase in the adrenal:body weight ratio. On the other hand, insulin sensitivity and blood sugar were not changed during that period of exposure. It is reasonable to conclude that the usual increase in the corticoadrenal activity was demonstrated as a result of this non-specific type of stress which is followed by no significant change in the carbohydrate metabolism. However, the corticoadrenal activity after 6 days of exposure was still very high and was accompanied by marked hyperglycemia and increased sensitivity to insulin. On the the 9th day when the animals reached a high degree of resistance to this non-specific stress, the blood sugar and insulin sensitivity were still at high levels.

The role of the anterior pituitary gland as the only part of the hypophysis responsible for maintenance for the normal resistance to insulin was indicated by Houssay and Patrick (1929), Pencharz *et al.* (1936), and Houssay (1942). Extirpation of the anterior lobe of the hypophysis was shown to increase the sensitivity to insulin. Recently the inclination has been toward the hypothesis that this function of the anterior pituitary is mainly due to an adrenocorticotrophic function. Adrenalectomized animals main-

tained by the administration of sodium salts are exceedingly liable to develop marked hyperglycemia or hypersensitivity to insulin after exposure to cold (Long, 1942). Recently, however, Cheng and Sayers (1949) demonstrated that the administration of DCA, by suppressing pituitary ACTH activity, produces a deficiency of glucocorticoids, with hypersensitivity to insulin. In our results, however, the increase in sensitivity to insulin on the 6th day, accompanied by apparent corticoadrenal hyperactivity, strongly suggests an increased inhibitory effect on the pituitary by the desoxycorticosterone-like substances. On the 9th day, further evidence is added to this hypothesis by the persistence of hypersensitivity to insulin while corticoadrenal activity approaches normal levels. The hyperglycemia observed on the 6th and 9th days of exposure to cold may be produced by adrenomedullary and other mechanisms not necessarily involving increased glucocorticoid output.

Thus both hyperglycemia and hypersensitivity to insulin could be maintained while indices of adrenocortical activity leveled off or approached normal levels. Furthermore, during adaptation to prolonged stress, the adrenocortico-chemical indices show recovery, while relative adrenal weight and glycemic indices still indicate stress.

SUMMARY

Adult rats were exposed to cold stress for one day, six days or nine days continuously. Blood sugar change due to insulin was measured before and after such exposure. The response of the adrenal glands of normal and stressed rats was determined by measurement of adrenal cholesterol, ascorbic acid and the ratio of total adrenal weight to body weight. Cold stress for one day, although it produced no significant change in blood sugar or insulin sensitivity, did cause marked depletion of adrenal cholesterol and ascorbic acid content. However, in the group exposed for 6 days there was hyperglycemia with hypersensitivity to insulin while the adrenal showed signs of higher activity. The rats exposed for nine days still had hyperglycemia and hypersensitivity to insulin although the adrenal activity returned near to normal levels. The results suggested that there is no direct correlation between corticoadrenal activity and sensitivity to insulin.

Acknowledgment

The authors wish to thank Dr. Edward W. Hawthorne, Acting Head of the Department of Physiology, for his advice and encouragement.

REFERENCES

Bloor, W. R., K. F. Palkan and D. M. Allen: *J. Biol. Chem.* **52**: 191. 1922.
Cheng, C. P. and G. Sayers: *Endocrinology* **44**: 400. 1949.

FISHER, R. A.: "Statistical methods for research workers" 11th ed. Edinburgh. Oliver and Boyde. 1950.
HO... AND D. PATRICK: *C.R. Soc. Biol.*, Paris **101**: 940. 1929.
HOUSSAY, B. A.: *Endocrinology* **30**: 884. 1942.
LONG, C. N. H.: *Endocrinology* **30**: 870. 1942.
LONG, C. N. H.: *Recent Prog. Hormone Res.* **1**: 99. 1947.
MASON, G.: *Endocrinology* **29**: 453. 1941.
PENCHARZE, R. T., C. F. CORI AND J. A. RUSSEL: *Proc. Soc. xp. Biol. Med.* **35**: 32. 1936.
SAYERS, M. A., G. SAYERS AND L. A. WOODBURY: *Endocrinology* **42**: 379. 1948.
SAYERS, G. AND M. A. SAYERS: *Rec. Prog. Hormone Res.* **2**: 81. 1948.
SELYE, H.: *Endocrinology* **21**: 169. 1937.
SELYE, H.: *J. Clin. Endocrinology* **6**: 117. 1946.
SHEPHERD, S. J., M. J. SMITH AND B. B. LONGWELL: *Endocrinology* **50**: 143. 1952.
ROE, J. H. AND C. A. KUETHER: *J. Biol. Chem.* **147**: 399. 1943.
VASSEUR, E.: *Acta Chem. Scand.* **2**: 693. 1948.

DEGENERATIVE JOINT DISEASE IN MICE BEARING ANTERIOR HYPOPHYSEAL, OVARIAN AND ADRENAL GRAFTS[1]

MARTIN SILBERBERG, RUTH SILBERBERG AND MARION OPDYKE

Snodgras Laboratory, City Hospital Division, and Department of Pathology, Washington University, School of Medicine, Saint Louis, Missouri

ENDOCRINE glands transplanted into closely related individuals may survive indefinitely and exert hormonal effects on various target organs (Loeb, 1945; Huseby and Bittner, 1948). In mice, ovarian and hypophyseal grafts stimulated mammary carcinogenesis and leukemogenesis (Loeb and Kirtz, 1939; Silberberg and Silberberg, 1950; Huseby and Bittner, 1951), and recently thyrotrophic activity of transplanted hypophyses has been demonstrated (Greer *et al.*, 1953). The method of transplantation seems to offer certain advantages over the injection of hormones: During long range experiments, there is apparently no loss of response as is seen with the administration of exogenous hormones, particularly of those of protein nature. Moreover, functional grafts may provide more uniform hormonal blood levels than obtainable with injected hormones.

In extension of previous investigations on skeletal changes produced by hormones (1942, 1952), the effects of homeotransplants of anterior hypophyses, ovaries and adrenals on degenerative joint disease of mice will presently be described.

MATERIAL AND METHODS

Male mice of the closely inbred strain A raised in our laboratory and kept on a stock diet of Purina Laboratory Chow and water *ad libitum* were used. Two sets of experiments were carried out: In one group of mice, orchiectomy was performed at the age of about 4 weeks, and in another group at the age of 1 to 3 days. For the study of mammary and adrenal growth, animals were sacrificed at various intervals. Since spontaneous degenerative joint disease does not occur in young mice, only those animals were included in the present paper which were, at the time of death, older than one year.

Received for publication May 19, 1953.

[1] The investigation was supported by research grant PHS A22(C2) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service, by a grant from the American Cancer Society on the recommendation of the Committee on Growth, National Research Council, and the Brant Fund of the American Medical Association.

The bones of the following animals were available: *First Group:* 22 untreated mice with intact testicles. *Second Group:* Mice orchiectomized at the age of four weeks: (a) 15 castrate controls; (b) 13 castrates that had received four anterior hypophyseal grafts; (c) 16 castrates that had received four ovarian grafts; (d) 22 castrates that had received two ovarian and four anterior hypophyseal grafts. *Third Group:* Mice orchiectomized soon after birth: (a) 31 castrate controls; (b) 22 castrates that had received four adrenal grafts; (c) 19 castrates that had received four adrenal and four anterior hypophyseal grafts.

In all animals, the endocrines were transplanted at the age of about one month. The grafts were made subcutaneously and taken from closely related donors 1 to 3 months of age. The technique of transplantation and the sex distribution of the donor animals have been reported previously; no sex differences in the effectiveness of the grafted hypophyses or adrenals could be established (1953).

At necropsy, care was taken to recover the transplants, and the removed tissues were identified microscopically. A number of grafts were fixed in Regaud's solution for cytologic studies, the results of which will be reported at a later date. The lower end of the femur and the upper end of the tibia including the kneejoint were removed as a whole, and fixed and decalcified in Bouin's solution. The material was embedded in paraffin, and semiserial sections stained with hematoxylin and eosin were prepared for microscopic studies.

OBSERVATIONS

1. *Fate of transplants*

The various grafts "took" readily. The changes occurring therein have been fully described elsewhere (1953). Presently it may suffice to state that in all mice sacrificed up to 4 months subsequent to transplantation surviving grafts were found. It may, therefore, be assumed that grafts in all

TABLE 1. SHOWING THE INCIDENCE OF DEGENERATIVE JOINT DISEASE

Experiment	Total No. of mice	Mean age of all mice (months)	Mice showing joint disease			No. of mice showing joint disease and surviving grafts
			No.	% of total	Mean age (months)	
I. Untreated controls	22	19.5	9	40.9	18.3	—
II.* a. Castrates	15	19.2	5	33.3	20.4	—
b. Castrates bearing hypophyseal grafts	13	18.0	8	61.5	18.1	5
c. Castrates bearing ovarian grafts	16	17.9	3	18.8	20.3	2
d. Castrates bearing hypophyseal and ovarian grafts	22	18.2	4	18.2	20.2	3[a]
III.† a. Castrates	31	18.8	7	22.6	18.2	—
b. Castrates bearing adrenal grafts	22	17.8	2	9.1	20.5	1
c. Castrates bearing adrenal and hypophyseal grafts	19	18.1	8	42.1	19.0	4[b]

* Orchiectomized at the age of about 4 weeks.
† Orchiectomized at the age of 1 to 3 days.
[a] Ovaries found in 3 mice, hypophyses in 2 of these 3.
[b] Adrenal and hypophyses found in all 4 of these.

cases were active for some time, even if they could not be recovered in old mice. Animals younger than 12 months do not appear in the table because joint disease was not seen before that age.

In half or more of the recipients which later developed degenerative

joint disease the grafts had remained alive until the end of the experiment. Representative surviving grafts are demonstrated in Figures 1–5.

2. *Findings in the kneejoints*

The usual architecture (Fig. 6) of and the age changes taking place in the articular tissues have been described previously (1952). The incidence of spontaneous osteoarthritis for various stains of mice and its increase up to the age of 2 years have also been reported (1952). Simple age changes were not considered in the present investigation. Typical articular lesions classifiable as hypertrophic or ulcerative types of degenerative joint disease are shown in figures 7 and 8. These changes may be considered as an analogue of human osteoarthritis. The incidence of the joint disease in animals of the various experimental groups is given in the table.

I. In untreated control mice, a 40.9% incidence of osteoarthritis was observed at a mean age of 18.3 months.

II. In mice orchiectomized at the age of 4 weeks, the incidence of osteoarthritis was 33.3% at a mean age of 20.4 months. This decrease, although numerically not impressive, becomes more significant in view of the fact that the mean age of these castrates was 2.1 months higher than that of the controls. The difference in mean age represents a critical period in the development of the articular lesions inasmuch as there is a marked increase in the incidence of the joint disease during this time. Of castrates bearing anterior hypophyseal grafts, 61.5% had osteoarthritis. Not only was the incidence of the disease higher, but also the mean age of these mice (18.1 months) was lower than that of the corresponding non-grafted castrates (33.3% at 20.4 months). The effect of the anterior hypophyseal grafts manifested itself not only in an increased incidence but also in an accelerated course of the joint disease. Conversely, the presence of ovarian transplants inhibited the development of the osteoarthritic changes: Of castrates bearing ovarian grafts (mean age 20.3 months) only 18.8% developed joint disease. In castrates of similar mean age (20.2 months), bearing

⟶

EXPLANATION OF FIGURES 1–5

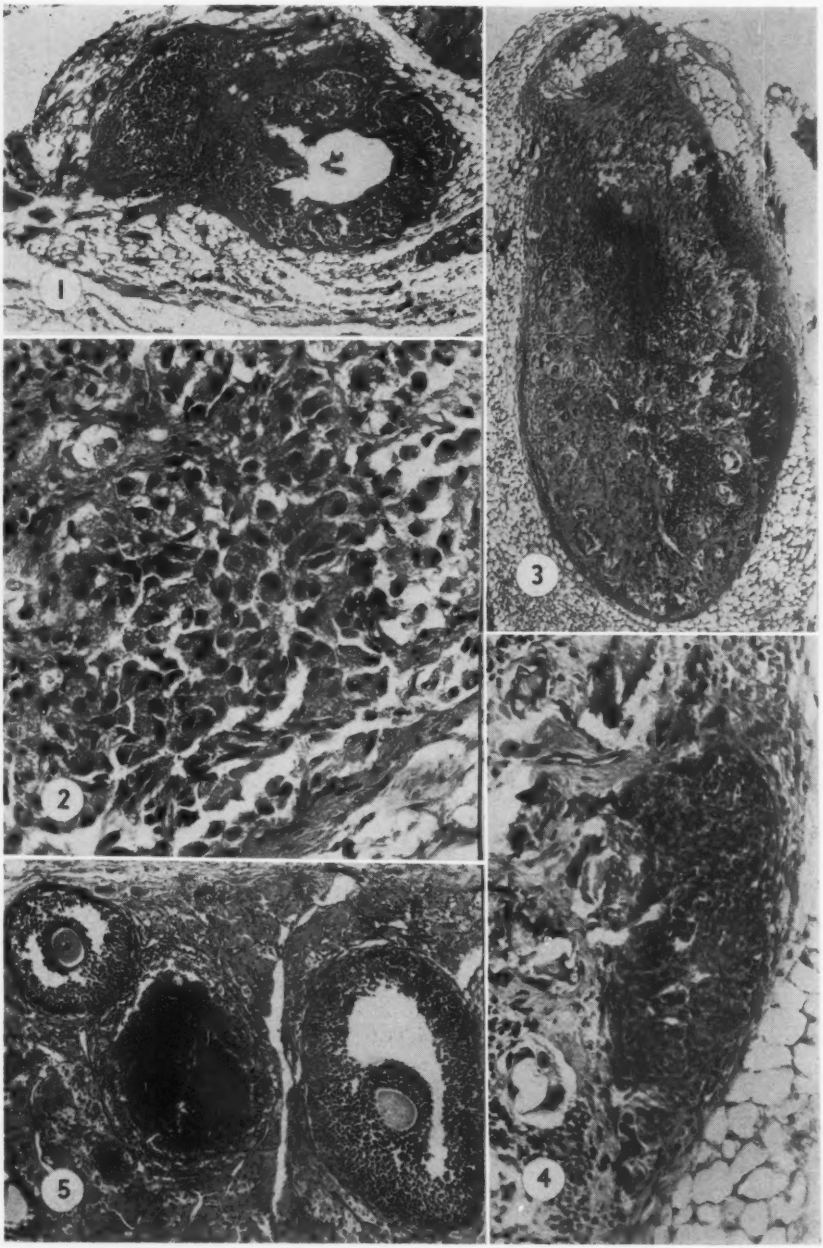
FIG. 1. Anterior hypophyseal graft 21 months after transplantation. ×170.

FIG. 2. Part of the graft shown in Fig. 1, showing well preserved cells of chromophobe type. ×345.

FIG. 3. Adrenal graft 10 months after transplantation. The mouse had received anterior hypophyseal grafts. There are areas of preserved cortical tissue with hyperplastic nodules. ×100.

FIG. 4. Hyperplastic cortical nodules present in the adrenal cortex of the graft shown in Fig. 3. ×300.

FIG. 5. Ovarian graft 14 months after transplantation. Mature follicles and ova are seen. ×93.


1
2
3
4
5

ovarian and anterior hypophyseal transplants, the observed incidence of articular lesions (18.2%) equalled that seen in mice bearing only ovarian grafts (18.8%). The hypophyseal transplants in animals of this group thus appeared to be ineffective as far as the articular cartilage is concerned.

III. Of mice orchiectomized soon after birth 22.6% had osteoarthritis at a mean age of 18.2 months. The incidence of the disease had thus dropped to about one half of that seen in animals of corresponding age (18.3 months) with intact testicles (40.9%). In castrates bearing adrenal grafts, the incidence of the articular lesions was further decreased to 9.1%. Since the mean age of these castrates was 20.5 months, that is 2.3 months higher than that of the untreated castrates of this group, the inhibiting effect of the adrenal secretions on the development of osteoarthritis was even more marked than would be indicated by the decreased incidence alone. By contrast, the stimulating effect of anterior hypophyseal grafts on the development of the joint disease was again illustrated by the findings in castrates bearing adrenal and anterior hypophyseal transplants: At a mean age of 19.0 months, these mice showed an almost fivefold increase in the incidence of degenerative joint disease (42.1%) over that seen in castrates bearing adrenal grafts alone (9.1%).

DISCUSSION

Besides the inherited rate of skeletal ageing and a number of exogenous factors (Bennett *et al.*, 1942), the pattern of endocrine secretions plays an important part in the evolution of degenerative joint disease (1952). The present results reemphasize the role of hormones in the pathogenesis of this disease.

Growth and degeneration of the articular cartilage are essential features of osteoarthritis, and hormones that modify these processes may alter the progress of the disease.

In mice orchiectomized before the onset of sexual maturity, skeletal growth and ageing are delayed, presumably owing to the lack of testosterone (1952). The retardation of these processes in the articular cartilage may then account for the decreased incidence of osteoarthritis observed in castrate mice. Whether or not castration performed after cessation of skeletal growth has the same effect as early castration is being investigated.

Anterior hypophyseal grafts intensified growth of the articular cartilage in castrates as they were shown to do in noncastrate animals (1942, 1952).

⟿

Explanation of Figures 6–7

Fig. 6. Unchanged articular surface. ×200.

Fig. 7. Osteoarthritic ulcers on the opposing surfaces of tibia and femur. Eburnation of the floor of the ulcers is seen. ×120.

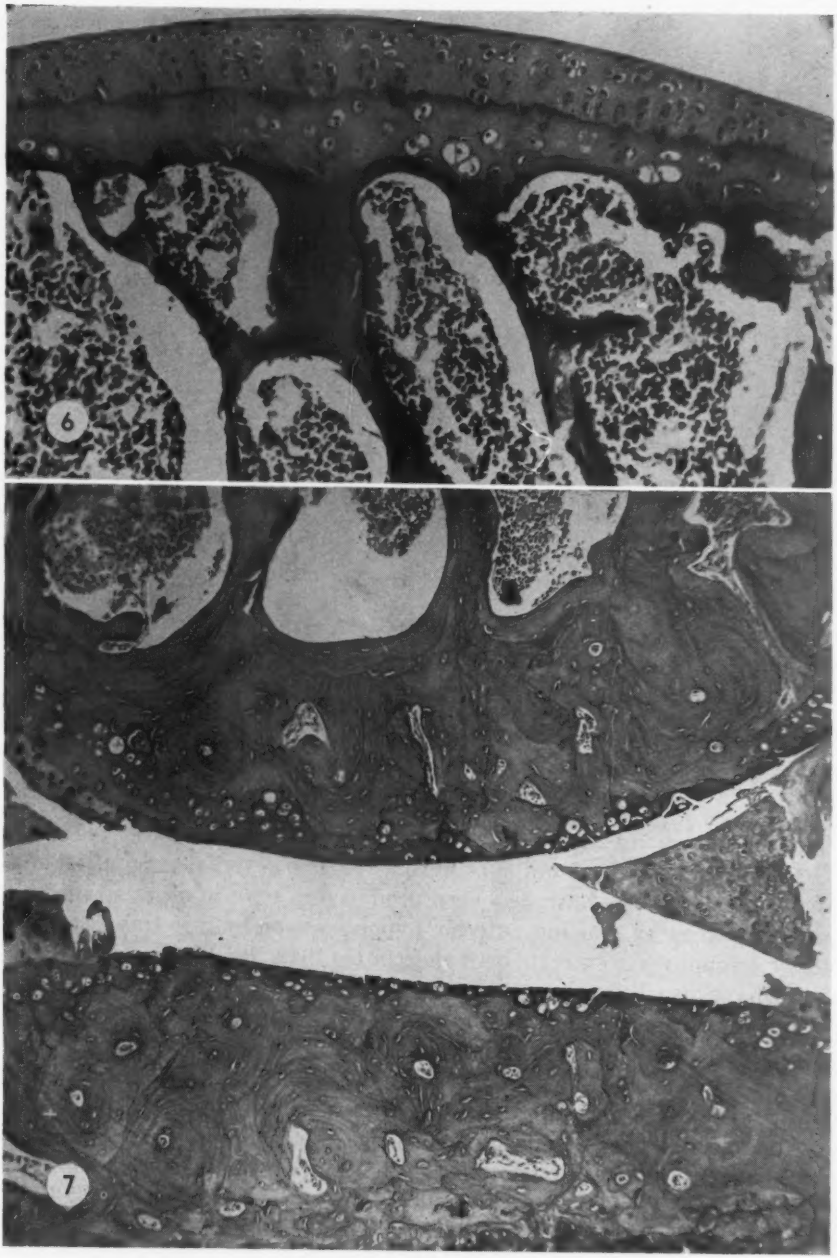

6
7

FIG. 8. Hypertrophic changes in the articular cartilage with cartilagination of the capsule. ×120.

This accentuation of growth was associated with or followed by regressive changes of cells and ground substance leading to severe degenerative joint disease. Such lesions have also been produced in mice of other strains and in guinea pigs by grafting anterior hypophyses or by injecting crude anterior hypophyseal extracts or *Antuitrin G* (1942, 1952); similar joint lesions have also been observed in rats receiving anterior hypophyseal extracts (Lucke and Hückel, 1933) and after treatment with anterior hypophyseal growth hormone (Reinhardt and Li, 1953). The severe arthropathic lesions seen in human acromegaly (Erdheim, 1931; Waine *et al.*, 1943) constitute extreme examples of the articular effects of hyperpituitarism.

Adrenal grafts decreased the incidence of osteoarthritis. This occurrence may be attributable to the production by the transplants of corticosteroids which are known to suppress somatic growth (Wells and Kendall, 1940; Baker, 1949; Ingle, 1950; Hench *et al.*, 1950; Follis, 1951), and therefore presumably also growth of the articular cartilage. The corticosteroids may

directly inhibit cell growth or they may primarily act on the ground substance and produce changes therein which in turn oppose growth. These effects of the adrenal grafts may be overcome by anterior hypophyseal transplants. In the presence of both types of grafts, the incidence of the joint disease was again increased. Apparently, the growth-promoting hormone given off by the transplanted hypophyses outbalanced not only adrenal activity but also any possible action of ACTH which might also have been produced by the hypophyseal grafts and which would be expected to exert a growth-inhibiting influence on the cartilage (Becks *et al.*, 1944; Ducommun, 1950).

Ovarian grafts decreased the incidence of osteoarthritis as did the adrenal transplants. This result may be correlated to the activity of estrogen produced by the grafted ovaries. Estrogen is known to inhibit growth and development of the cartilage (1952), and this hormone would therefore counteract the development of osteoarthritis.

In the presence of ovarian grafts, hypophyseal transplants failed to exert their typical growth-promoting effect on the articular cartilage, and the incidence of degenerative joint disease was not increased over that seen in animals bearing ovarian grafts only. This occurrence may be explained, as follows: Under the influence of the hypophyseal grafts, the transplanted ovaries give off excessive amounts of estrogen which prevent the growth-promoting hormone of the grafted hypophyses to assert itself. That a state of hyperestrinization existed in the mice of this group was indicated by the intensification of mammary growth including the production of cancer, the increased incidence of leukemia, and the typical condensation in the shafts of the long bones.

SUMMARY

In male mice of strain A, orchiectomy decreased the incidence of degenerative joint disease. Homeotransplants of adrenals or ovaries further inhibited the development of the articular lesions. Anterior hypophyseal transplants increased the incidence and accelerated the onset of the joint disease. The effects of grafted hypophyses on the joints were counteracted by transplanted adrenals or ovaries, the ovaries being more effective in this respect than the adrenals.

REFERENCES

Baker, B. L.: Pituitary-Adrenal Function. Amer. Assoc. Advanc. Science, 88, 1949.

Becks, H., M. E. Simpson, W. Marx, C. H. Li and H. M. Evans: *Endocrinology* **34**: 311. 1944.

Bennett, G. A., H. Waine and W. Bauer: Changes in the Kneejoint at Various Ages; With Particular Reference to the Nature and Development of Degenerative Joint Disease. New York, Commonwealth Fund, 1942.

DUCOMMUN, P.: *Acta endocrinol.* **4:** 343. 1950.
ERDHEIM, J.: Die Lebensvorgaenge im normalen Knorpel und seine Wirkung bei Akromegalie. Berlin, Julius Springer, 1931.
FOLLIS, R. H. JR.: *Proc. Soc. Exp. Biol. & Med.* **78:** 723. 1951.
GREER, M. A., R. O. SCOW AND C. GROBSTEIN: *Proc. Soc. Exp. Biol. & Med.* **82:** 28. 1953.
HENCH, P. S., E. C. KENDALL, C. H. SLOCUMB AND H. F. POLLEY: *Arch. Intern. Med.* **85:** 545. 1950.
HUSEBY, R. A. AND J. J. BITTNER: *Proc. Soc. Exp. Biol. & Med.* **69:** 321. 1948.
HUSEBY, R. A. AND J. J. BITTNER: *Cancer Research* **11:** 450. 1951.
INGLE, D. J.: *J. Clin. Endocrinol.* **10:** 1312. 1950.
LOEB, L. AND M. M. KIRTZ: *Amer. J. Cancer* **36:** 56. 1939.
LOEB, L.: The Biological Basis of Individuality. Springfield, Ill., Charles C Thomas, 1945.
LUCKE, H. AND R. HUECKEL: *Arch. f. Exper. Pathol. und Pharmakol.* **169:** 290. 1933.
REINHARDT, W. O. AND C. H. LI: *Science* **151:** 295. 1953.
SILBERBERG, M. AND R. SILBERBERG: *Endocrinology* **31:** 410. 1942.
SILBERBERG, M. AND R. SILBERBERG: *Arch. Pathol.* **49:** 733. 1950.
SILBERBERG, M. AND R. SILBERBERG: In Rheumatic Diseases, p. 151, W. B. Saunders & Co., Philadelphia and London, 1952.
SILBERBERG, R., M. SILBERBERG AND M. OPDYKE: *Arch. Pathol.* **55:** 506. 1953.
WAINE, H., G. A. BENNETT AND W. BAUER: *Amer. J. Med. Science* **209:** 671. 1945.
WELLS, N. B. AND E. C. KENDALL: *Proceed., Mayo Clin. Staff Meet.* **15:** 324. 1940.

A THYROID MODEL DESCRIBING KINETICS OF EXCHANGE, CONCENTRATING, AND ORGANIC BINDING OF IODIDE

SEYMOUR H. WOLLMAN

Laboratory of Biophysics, National Cancer Institute,[1] Bethesda 14, Maryland

IN MODELS describing the kinetics of accumulation of radioiodine by thyroid tissue it has been assumed that the equilibration of radioiodide between blood and thyroid tissue is instantaneous (Myant, Pochin and Goldie, 1949; Oddie, 1949; Brownell, 1951; Riggs, 1952; Wollman, 1953). However, recent measurements show that the equilibration is not instantaneous in the mouse (Wollman and Scow, 1953). In addition, the following simple calculation shows that the equilibration must be expected to take an appreciable length of time if the ratio of the radioiodide concentrations in thyroid gland and serum (T/S ratio) is large. The T/S ratio has been found to be approximately 250 in the normal mouse (Wollman and Scow, 1953). This implies that at equilibrium the radioiodide content of the thyroid is 250 times that of an equal volume of serum or (approximately) blood.[2] If one assumes that the radioiodide concentration in the blood of the mouse is held constant after injection, a volume of blood 250 times the thyroid volume must flow through the thyroid in order to establish equilibrium if all the radioiodide is removed from the perfusing blood. The time required for this would be 25 minutes if one assumes a rate of volume flow of 10 volumes of blood per volume of thyroid per minute (Riggs, 1952).

A new model has therefore been developed[3] by means of which it has

Received for publication May 30, 1953.

[1] National Institutes of Health, Public Health Service, Department of Health, Education and Welfare.

[2] The radioiodide concentration in the blood is probably slightly less than in serum. Although data for the comparison between blood and serum do not seem to be available, comparisons are available between blood and plasma. Riggs (1952) estimates the iodide concentration in blood to be 20 per cent less than in plasma. It is unfortunate that so many data on blood are published in terms of the composition of serum or plasma. From the theoretical point of view it would be desirable to have data on the composition of the non-cellular portion of circulating blood (which may be different from that of plasma as conventionally prepared) or of whole blood itself, depending upon the question being considered.

[3] Kety (1951) has written a stimulating summary of the analogous problem of the exchange of inert gases between blood and tissues.

been possible to make an analysis of the effects of the time interval required for equilibration on certain aspects of thyroidal iodine metabolism. A qualitative comparison has been made between predictions of the theory and some experimental data, but no attempts have been made to fit experimental curves or to compute values of constants mostly because of the absence of suitable data.

MODEL

In the derivations to be made attention is focused on the thyroid gland and its blood supply. All other organs of the animal are ignored or taken account of only indirectly. The model considers transport of iodide to the thyroid, exchange of iodide between the blood and the thyroid gland, and organic binding of iodide. Iodide is to be transported to the thyroid and removed from it only by the blood. Transport by lymph is neglected.

The animal is assumed to be in iodide balance. The stable iodide concentrations in its thyroid gland and arterial blood are both to be constant. In addition, the stable iodide concentration is to be low enough so that the iodide does not either inhibit organic binding or depress the T/S ratio. The injected radioiodide is to be carrier-free. The concentration of radioiodide in the blood is assumed to be either constant or to decrease exponentially with time after injection. The latter case is selected to take care of a single intravenous injection experiment; removal of injected radioiodide from the blood by all the tissues is corrected for by the choice of exponent.

The unit of functional activity of the thyroid gland is assumed to be the follicle. All follicles are assumed to be arranged in parallel (Major, 1909); blood in the capillary net around a follicle exchanges with only one follicle. All follicles are assumed equivalent in that the same volume of blood circulates around each follicle per unit time per unit weight of tissue, and the follicles are to have the same ratio of tissue iodide concentration to blood iodide concentration.

Exchange of iodide between blood and thyroid tissue is pictured as follows: Each volume element of blood entering the thyroid gland breaks up into sub-units, one to each follicle. The follicles, for simplicity, are thought of as points. From each volume element of blood a fixed number of iodide ions or a constant fraction of the stable iodide in the blood is transferred to thyroid tissue; and this is replaced by a constant but equal or smaller number of iodide ions from the thyroid tissue depending upon whether organic binding is blocked or permitted, respectively. The fraction transferred is actually determined by factors analogous to those in the phenomenon of diffusion, i.e., the time interval required for blood to flow through the capillary net around the follicle, length of capillary, surface area per unit length of capillary, and permeability characteristics of the capillaries and

parenchymal cells (mechanism of the transport process), but such details are ignored for the present. The point follicle is a good approximation for either of the following special cases: 1) The stable iodide and radioiodide concentrations in the blood do not change very much as the blood perfuses the follicle. 2) Once iodide is transferred to the thyroid tissue, equilibration of iodide in the iodide space of the follicle is instantaneous.

The thyroid iodide considered in this analysis is iodide which is freely exchangeable with blood iodide; it is the only iodide which can become labeled rapidly and which can play an important part in radioiodide uptake. When organic binding of iodide occurs it is assumed that the iodide being bound is thyroid iodide with specific activity (if radioiodide is present) characteristic of freely exchangeable thyroid iodide and not of blood iodide. Organic binding of iodide by the thyroid is assumed to be a first order reaction as suggested by the work of Stanley (1949) on man.

The processes of iodide concentrating and iodide binding are assumed to be strictly independent of each other (Wollman, Scow, and Morris, 1953) except in so far as they involve a substance common to both processes. This assumption appears justified since each of the two processes can be independently inhibited by certain goitrogens (Pitt-Rivers, 1950) and since each process seems to occur in the apparent absence of the other in certain thyroid tissues (Wollman, Scow and Morris, 1953; Wollman and Zwilling, 1953). When binding is blocked by a goitrogen, it is assumed that the sole direct effect of the goitrogen is to prevent organic binding of iodide.[4]

SYMBOLS

- B Blood (Subscript).
- c Rate of volume flow of blood through thyroid gland in ml/min.
- C_T Blood iodide clearance by thyroid.
- F Fraction of blood iodide transferred to thyroid tissue as blood perfuses the thyroid.
- F_b Fraction of the amount of stable blood iodide entering thyroid iodide space which becomes organically bound.
- F_E Ratio of the change in the blood iodide concentration to the arterial iodide concentration as the blood perfuses the thyroid = Extraction ratio.
- I Iodide concentration in the tissue indicated by the subscript.
- I_0^* Initial concentration of blood radioiodide.
- K Rate constant for decrease of the radioiodide concentration in the blood.
- K_b Rate constant for organic binding of thyroid iodide.

---

[4] This assumption is not used in the derivations. Its validity is important in order that the derived formulae be applicable to predicting the effect on the T/S ratio of blocking binding with a goitrogen. If a goitrogen has a direct effect on iodide concentrating or other processes in addition to the effect on binding, the formulae can be expected to predict only that part of the effect on the T/S ratio due to the blocking of binding.

K_e, K_{be} Rate constant for equilibration of iodide between blood and thyroid gland when organic binding is blocked or permitted, respectively.
Q_b Quantity of iodide bound by thyroid gland.
R, R_b Ratio of stable iodide concentration in thyroid gland and blood when organic binding is blocked or permitted, respectively.
t Time.
T Thyroid (Subscript).
v Rate of volume flow of blood through thyroid gland per unit volume of gland.
V Volume.
* Radioactive (Superscript).

Effect of organic binding on the ratio of the stable iodide concentrations in thyroid gland and blood

Blood containing iodide at a concentration, I_B, flows through the thyroid gland. From each volume element of this blood, ΔV_B, a fraction of the iodide, F, goes into the thyroid so that the amount of iodide going into the gland is $I_B F \Delta V_B$. If no organic binding occurs, at equilibrium the concentration of iodide in the thyroid, I_T, is given by $I_T = RI_B$ where R is the equilibrium ratio of the stable iodide concentrations in thyroid gland and blood. Since at equilibrium the thyroid iodide concentration is a constant the amount of iodide leaving the thyroid must equal that entering. It is assumed that if equilibrium is not established the amount of iodide leaving the gland and going into ΔV_B is proportional to the concentration of thyroid iodide, i.e., the amount leaving at equilibrium (when the thyroid iodide concentration is RI_B) is $I_B F \Delta V_B$. The amount leaving for unit concentration is then

$$\frac{I_B F \Delta V_B}{RI_B} = \frac{F \Delta V_B}{R}$$

and the amount leaving when the thyroid iodide concentration is I_T is $(I_T/R)F\Delta V_B$. The change of the iodide concentration in the thyroid will be given by the difference in amounts entering and leaving the gland, divided by the thyroid volume, V_T. Assuming $V_B = c\Delta t$, where c is the rate of volume flow of blood through the gland in ml/min., the change in thyroid iodide concentration is given by

$$\Delta I_T = \frac{1}{V_T}\left(I_B F \Delta V_B - \frac{I_T}{R} F \Delta V_B\right) = \left(I_B - \frac{I_T}{R}\right)\frac{cF}{V_T}\Delta t$$

or

$$\frac{dI_T}{dt} = (RI_B - I_T)\frac{cF}{RV_T} = K_e(RI_B - I_T) \qquad (1)$$

where K_e is a rate constant for equilibration.

This equation states that the rate of increase of the thyroid iodide concentration is proportional to the difference between the thyroid iodide concentration at equilibrium and the concentration existing at that moment since $RI_B = I_T$ at equilibrium.

When organic binding of iodide occurs in the thyroid, it is assumed that the rate of binding is proportional to the iodide concentration in the gland, i.e.,

$$\frac{dI_T}{dt} = -K_b I_T = -\frac{1}{V_T}\frac{dQ_b}{dt} \tag{2}$$

where K_b is the rate constant for organic binding of iodide in the gland. If both binding and equilibrating of iodide between thyroid gland and blood occur the rate of increase of the concentration of thyroid iodide will be given by the difference between the rate of increase of the thyroid iodide concentration due to net transfer of iodide from the blood, and the rate of decrease due to binding:

$$\frac{dI_T}{dt} = (RI_B - I_T)\frac{cF}{RV_T} - K_b I_T. \tag{3}$$

Since for a steady state $dI_T/dt = 0$, the thyroid iodide concentration will be a constant given by

$$I_T = \frac{I_B R K_e}{K_b + K_e} = \frac{I_B R}{1 + \frac{K_b}{K_e}} = I_B R_b \tag{4}$$

R_b is the ratio of stable iodide concentrations in thyroid gland and blood when binding is allowed.

Eq. (4) indicates that the ratio of the stable iodide concentrations in thyroid gland and blood is expected to be smaller, the larger the binding rate constant relative to the rate constant for equilibration of iodide between blood and thyroid gland.

Exchange and concentrating of radioiodide

Because of the difficulty in making accurate stable iodide analyses, and because the concentration of the stable iodide in the blood of some species is so low that it usually has not been possible to measure it directly (Taurog, Tong and Chaikoff, 1951), radioiodide has been used to make estimates of the ratio of the concentrations of stable iodide in thyroid gland and blood.

In the following paragraphs a derivation is made of a formula describing the course of the equilibration of injected radioiodide between blood and thyroid gland. The formula obtained describes how the relation between R and the ratio of the radioiodide concentrations in thyroid gland and blood depends upon certain other variables. In addition formulae will be derived which describe the rate of organic binding of radioiodide by the thyroid, and the time dependence of the extraction ratio during the period of equilibration.

When a volume element of blood, ΔV_B, passes through the thyroid gland a constant fraction, F, of its stable iodide passes into the tissue; the amount of stable iodide entering the tissue is $I_B F \Delta V_B$. When this happens an amount of radioactive iodide

$$\frac{I_B^*}{I_B} \cdot I_B F \Delta V_B = I_B^* F \Delta V_B$$

also enters the tissue (where I_B^*/I_B is the specific activity of the iodide in the blood). The iodide from the blood which enters the thyroid tissue mixes with the iodide already present in the tissue to give an iodide concentration $I_T+(I_BF\Delta V_B/V_T)$ in the tissue with specific activity

$$\frac{I_T^* + \frac{I_B^* F \Delta V_B}{V_T}}{I_T + \frac{I_B F \Delta V_B}{V_T}} = \frac{I_T^*}{I_T}$$

which is essentially unchanged in the first approximation when ΔV_B is made very small relative to V_T.

A fraction of the amount of iodide which has been transferred from ΔV_B to thyroid tissue, F_b, namely, an amount of thyroid iodide given by $Q_b=F_bI_BF\Delta V_B$ is bound, and, accompanying this, an amount of radioiodide which equals

$$Q_b^* = \frac{I_T^*}{I_T} F_b I_B F \Delta V_B = \frac{I_T^*}{R_b} F F_b \Delta V_B \tag{5}$$

is bound. The remainder of the iodide from ΔV_B, $(1-F_b)I_BF\Delta V_B$, with specific activity now equal to that of iodide in the thyroid tissue, and an amount of radioiodide equal to

$$\frac{I_T^*}{I_T}(1 - F_b) I_B F \Delta V_B = \frac{I_T^*}{R_b}(1 - F_b) F \Delta V_B$$

are returned to ΔV_B.

The change in the concentration of radioiodide in the thyroid due to exchange of thyroid iodide with iodide in ΔV_B equals the difference in the amount of radioiodide entering, $I_B^*F\Delta V_B$, and the amount leaving the thyroid iodide space,

$$\frac{I_T^*}{R_b} F F_b \Delta V_B + \frac{I_T^*}{R_b}(1 - F_b) F \Delta V_B = \frac{I_T^*}{R_b} F \Delta V_B,$$

due to transfer back to blood or to binding, respectively, divided by the thyroid volume, V_T.

That is

$$\Delta I_T^* = \left(I_B^* F \Delta V_B - \frac{I_T^*}{R_b} F \Delta V_B \right) \frac{1}{V_T} = (R_b I_B^* - I_T^*) \frac{F \Delta V_B}{R_b V_T}.$$

Now if $I_B^*=I_0^*e^{-Kt}$ and $\Delta V_B=c\Delta t$ the last equation becomes

$$\frac{dI_T^*}{dt} = (R_b I_0^* e^{-Kt} - I_T^*) \frac{cF}{R_b V_T} = (R_b I_0^* e^{-Kt} - I_T^*) K_{be}$$

where $K_{be}=cF/R_bV_T$ or

$$\frac{dI_T^*}{dt} + K_{be} I_T^* = K_{be} R_b I_0^* e^{-Kt}. \tag{6}$$

This equation has as its integral, if $I_T^*=0$ when $t=0$,

$$I_T^* = \frac{R_b I_0^*}{1 - \frac{K}{K_{be}}} (e^{-Kt} - e^{-K_{be}t}). \tag{7}$$

The ratio of the radioiodide concentrations in thyroid gland and blood is then given by

$$\frac{I_T^*}{I_B^*} = \frac{R_b}{1 - \frac{K}{K_{be}}} (1 - e^{-(K_{be}-K)t}). \tag{8}$$

The corresponding equation for the case in which binding is blocked ($K_b=0$) differs from eq. (8) only in that K_{be} and R_b are replaced by K_e and R, respectively.

This equation indicates that a steady state value of I_T^*/I_B^* can be obtained only if K is less than K_{be}. If a steady state is obtained I_T^*/I_B^* must be expected to be not equal to but greater than the corresponding ratio of stable iodide concentrations, R, unless the rate constant for decrease of the radioiodide concentration in blood is small compared with the rate constant for equilibration.

In the mouse in which organic binding of iodide by the thyroid is blocked this effect is probably negligible since the rate of loss of radioiodide from the blood appears small enough[5] (Wollman and Scow, 1953). However, to insure simple interpretation of measurements of T/S ratios using radioiodide it is necessary to compare the rate of decrease of the radioiodide concentration in the blood with the rate of equilibration of radioiodide between blood and thyroid gland. It appears to be especially important to check this in experimental animals in which the equilibration rate constant may be less than normal (for example, after hypophysectomy (Wollman and Scow, 1953)), or in which the rate constant for loss of radioiodide from the blood might be elevated (if clearance of blood iodide by the thyroid or kidney were elevated).[6]

If the rate of loss of radioiodide from the blood can be neglected, $K \ll K_{be}$, eq. (8) becomes

$$\frac{I_T^*}{I_B^*} = R_b(1 - e^{-K_{be}t}). \tag{8a}$$

Radioiodide concentrating can then be described by two constants K_{be} (or K_e) and R_b (or R). K_e is given by vF/R where $v=c/V_T$. It may be possible to determine v by measuring the arteriovenous difference of iodide concentrations in blood perfusing the thyroid along with I^{131} content in the thyroid at various time intervals after radioiodide injection (Kety, 1949). F can be calculated when v, R and K_e are known.

The values of the constants R (or R_b), K_e, v and F might depend upon whether binding is allowed or blocked. So far a direct experimental comparison has been made only for R (Wollman and Scow, 1953) and it has

[5] Recent unpublished observations in mice (Wollman and Scow) indicate that at high enough doses of propylthiouracil (5 mg. per 25 gm. body weight) the rate of decrease of the radioiodide concentration in the blood becomes high enough to influence the T/S ratio by the above mechanism.

[6] Considerations analogous to the above have been used by Smith (1951) in pointing out the difficulty in estimating kidney clearance of a substance from the blood on the basis of measurements of the rate of decrease of the concentration of the substance in the blood.

been found in the hypophysectomized mouse that R is smaller when binding is allowed than when blocked (in accordance with predictions in this paper). From this analysis it is also to be expected that if other factors remain constant the equilibration rate constant, K_e, will be larger when binding is allowed than when blocked, since K_e varies inversely as R.

If organic binding is blocked and radioiodide has equilibrated between thyroid gland and blood it is easy to show using eq. (6) that if radioiodide be suddenly removed from the thyroid arterial blood the thyroid radioiodide concentration would be expected to decrease exponentially as in the equation

$$I_T^* = RI_0^* e^{-(vF/R)t} \tag{9}$$

where RI_0^* is the initial radioiodide concentration in the thyroid. The rate constant for loss of radioiodide would therefore be expected to be the same as for gain.

In contrast, if administration of thiocyanate causes a loss of iodide from the thyroid gland (because R is suddenly reduced to approximately one), using eq. (1), the thyroid iodide concentration can be shown to be given by

$$I_T = I_B + I_B(R - 1)e^{-vFt}. \tag{10}$$

The rate constant $K_e = vF$ is larger than that in the preceding case by a factor of R. The difference appears to arise from the fact that a ratio of R restricts the iodide concentration in the blood to be $1/R$ times that in the thyroid gland while iodide is being removed. It is therefore not proper to conclude from the observed rapid loss of thyroid iodide after administration of thiocyanate (Stanley and Astwood, 1948; Stanbury and Wyngaarden, 1952), that equilibration of radioiodide is normally rapid.

Organic binding of radioiodide

The amount of radioiodide bound during exchange of iodide between ΔV_B and the thyroid was given by eq. (5):

$$\Delta Q_b^* = \frac{I_T^*}{R_b} FF_b \Delta V_B.$$

Using $\Delta V_B = c\Delta t$ and eq. (7) for I_T^*, this yields the differential equation for accumulation of bound radioiodine

$$dQ_b^* = \frac{FF_b I_0^* c}{1 - \frac{K}{K_{be}}} (e^{-Kt} - e^{-K_{be}t})dt. \tag{11}$$

On integrating, assuming $Q_b^* = 0$ when $t = 0$, this yields

$$Q_b^* = \frac{FF_b I_0^* c}{1 - \frac{K}{K_{be}}} \left[\frac{1}{K} (1 - e^{-Kt}) + \frac{1}{K_{be}} (e^{-K_{be}t} - 1) \right]. \tag{12}$$

A case of this equation, of special interest because of its simplicity, is

that in which the radioiodide concentration in the blood is held constant ($K=0$). When t is sufficiently large, eq. (12) becomes

$$Q_b^* = FF_bI_0^*c\left(t + \frac{1}{K_{be}}\right) \tag{12a}$$

Eq. (12a) implies that Q_b^* will increase linearly with the time at large t and, when this linear portion is extrapolated to $t=0$, will give an intercept on the t-axis of $Q_b^* = (FF_bI_0^*c/K_{be})$. If equilibration were instantaneous, $K_{be} = \infty$, and Q_b^* vs. t would go through the origin.

Thyroid arteriovenous difference in radioiodide concentrations

The concentration of radioiodide in thyroid venous blood is given by

$$I_B^* - FI_B^* + (1 - F_b)\frac{FI_T^*}{R_b}$$

where the first term is the arterial radioiodide concentration, the second is the concentration change due to transfer of blood radioiodide to the thyroid and the third is the concentration change due to transfer of thyroid radioiodide to blood.

Upon substituting $I_B^* = I_0^*e^{-Kt}$ and eq. (7) for I_T^*, this yields

$$(1 - F)I_0^*e^{-Kt} + \frac{(1 - F_b)FI_0^*}{1 - \frac{K}{K_{be}}}(e^{-Kt} - e^{-K_{be}t}).$$

The difference between the arterial and the venous concentrations is given by

$$\Delta I_B^* = FI_0^*e^{-Kt} - \frac{(1 - F_b)FI_0^*}{1 - \frac{K}{K_{be}}}(e^{-Kt} - e^{-K_{be}t}).$$

The thyroid extraction ratio [$F_E = (\Delta I_B/I_B)$] is given by

$$F_E = F - \frac{(1 - F_b)F}{1 - \frac{K}{K_{be}}}(1 - e^{-(K_{be}-K)t})$$

or

$$F_E = \frac{F_bF - \frac{FK}{K_{be}} + F(1 - F_b)e^{-(K_{be}-K)t}}{1 - \frac{K}{K_{be}}}. \tag{13}$$

There are several special cases of interest. In all of them, however, when $t=0$, $F_E=F$. That is, just after radioiodide injection the extraction ratio is limited by the fraction of the blood iodide which exchanges with thyroid iodide. After equilibration the value of the extraction ratio depends upon the case. For example, after equilibration of radioiodide, if the radioiodide concentration in the blood is constant and organic binding is permitted,

$F_E = FF_b$. The extraction ratio will therefore be expected to decrease during the equilibration and to become constant after equilibration with a value given by the fraction of the blood radioiodide which becomes bound.

Factors controlling radioiodine accumulation

After equilibration, the radioiodine uptake is controlled by the rate at which stable iodide is bound (eq. 2). The binding rate of radioiodine is given by the product of the volume of tissue, the binding rate constant and the thyroid radioiodide concentration.

$$\frac{dQ_b^*}{dt} = V_T K_b I_T^*.$$

Substituting for I_T^* from

$$\frac{I_T^*}{I_B^*} = \frac{R_b}{1 - \frac{K}{K_{be}}}$$

(eq. (8) after equilibration) the above equations yields

$$\frac{dQ_b^*}{dt} = \frac{V_T K_b R_b I_B^*}{1 - \frac{K}{K_{be}}} = \frac{V_T K_b R I_B^*}{\left(1 - \frac{K}{K_{be}}\right)\left(1 + \frac{K_b}{K_e}\right)}.$$

Conventionally, the rate of accumulation of radioiodine has been written as a product of a clearance constant, C_T, and the blood concentration of radioiodide, I_B^*, i.e.,

$$\frac{dQ_b^*}{dt} = I_B^* C_T.$$

Comparison of the last two equations indicates that an explicit expression for the thyroid clearance is given by

$$C_T = \frac{V_T K_b R}{\left(1 - \frac{K}{K_{be}}\right)\left(1 + \frac{K_b}{K_e}\right)} = \frac{V_T K_b R}{\left(1 + \frac{K_b R}{vF}\right)\left(1 - \frac{K}{K_{be}}\right)} \qquad (14)$$

since $K_e = vF/R$.

Two special cases of eq. (14) are of interest. For simplicity in the discussion K will be assumed to be small compared with K_{be}.

1) The rate constant for equilibration, K_e, is small compared with the binding rate constant $K_b (K_e \ll K_b)$. This situation can be recognized as the one in which

$$R_b = \frac{R}{1 + \frac{K_b}{K_e}} \ll R.$$

The thyroid clearance is given by

$$C_T = V_T K_e R = V_T v F = cF. \tag{14a}$$

The thyroid clearance, C_T, is expected to be relatively independent of K_b and R; the rate of delivery of iodide from the blood to the thyroid gland will control the binding rate or the thyroid clearance. This appears to be the situation for the thyroid gland of the hypophysectomized mouse (Wollman and Scow, 1953).

2) The rate constant for equilibration is large compared with the binding rate constant ($K_e \gg K_b$). This situation can be recognized as the one in which

$$R_b = \frac{R}{1 + \dfrac{K_b}{K_e}} = R.$$

The thyroid clearance is given by

$$C_T = V_R K_b R. \tag{14b}$$

The thyroid clearance is expected to be independent of the rate of delivery of iodide to the thyroid. This seems to be the case for the thyroid gland in man, since Ingbar (1952) has obtained data indicating that in man that T/S ratio is independent of whether or not binding is blocked.

DEVIATIONS FROM THE MODEL

1. Non-equivalence of all thyroid iodide: At the present time there appears to be some evidence for three different iodide compartments in the thyroid gland: a) Iodide which is not freely exchangeable with iodide in the blood.[7] The analysis in this paper does not take this iodide (if it exists) into account; this iodide would not take part in uptake of radioiodide; b) iodide which is freely exchangeable with blood iodide and which is located in a site where binding occurs and whose concentration determines the rate of organic binding; c) iodide which is freely exchangeable with blood iodide, but which is located at a site where binding does not occur.

The concentration of iodide in this latter site may not be of importance ordinarily since it may be negligible compared with the total concentration of freely exchangeable iodide in the thyroid gland. However, in the special case of transplantable tumors of the thyroid gland a few tumor lines have

[7] Evidence for this compartment: the ratio of the stable iodide concentration in the thyroid to that in plasma (Taurog, Tong and Chaikoff, 1951) is higher than the corresponding ratio for freely-exchangeable iodide as determined using radioiodide and thiouracil.

been tested in which the total freely exchangeable iodide concentration was not much higher than that expected from iodide confined largely to extracellular space (Wollman, Scow and Morris, 1953). In this case it appears likely that the equation concerned with the rate of organic binding must be modified so that the rate is proportional not to the total radioiodide concentration in the gland, but to the radioiodide concentration at the site of the binding.

2. Non-uniformity in size and rate of blood flow in various follicles: Where non-uniformity exists the curve of the T/S ratio as a function of the time after radioiodide injection may be modified depending upon the characteristics of the population of follicles. The modification will always be in the direction of an initially steeper curve which will deviate from linearity earlier and continue to rise for a longer time than predicted from $I_T^*/I_B^* = R(1-e^{-K_e t})$.

3. The fraction of the blood iodide, F, which is transferred from blood to thyroid tissue is large (approximately 1): In the derivations the iodide or the radioiodide concentration of the blood is assumed implicitly not to change appreciably as the blood flows through the thyroid gland. If F is close to one, however, this is not true, and it is necessary to take into account the fact that the blood radioiodide content decreases significantly and progressively as the blood flows through the capillary net around the follicle. This has the effect of making the equilibration more rapid than predicted from $I_T^*/I_B^* = R(1-e^{-K_e t})$ by making I_T^*/I_B^* proportional to the time for greater values of I_T^*/I_B^* than predicted from the exponential relation. An analysis (similar to that which has been presented) for the case that $F = 1$ and the blood iodide exchanges on the average several times during passage around the follicle is in progress.

4. If some iodide in the thyroid is normally released from protein bound thyroid iodine by mechanisms involving desiodinating enzymes (Roche, Michel, Michel and Lissitzky, 1952) at a rate approximating the rate of transport of iodide from blood to thyroid tissue, the above model will have to be modified. This possibility appears to have been ignored in all previous models and unfortunately must be omitted here because of lack of data.

5. If arteriovenous shunts are important, i.e., if a significant fraction of the arterial blood going to the thyroid by-passes the follicles but later combines with thyroid venous blood from the follicles, direct measurements of F will underestimate its true value (Kety, 1951).

SUMMARY

Using a simple model, equations have been obtained describing exchange, concentrating, and organic binding of iodide by the thyroid gland. The analysis indicates that when the ratio of the iodide concentrations in

thyroid gland and serum is large enough, equilibration of radioiodide between thyroid gland and blood may take an appreciable time. Whether equilibration of radioiodide between thyroid gland and blood can be considered instantaneous depends not upon the magnitude of the rate of equilibration, but upon its magnitude compared with a) the rate of decrease of the radioiodide concentration in the blood and b) the rate of organic binding of thyroid iodide. If equilibration cannot be considered instantaneous the following consequences are predicted: 1) If the rate of organic binding of thyroid iodide is large enough compared with the rate of equilibration of iodide between thyroid gland and blood, the ratio of the thyroid iodide concentration to the blood iodide concentration will be greater when organic binding is blocked than when permitted; 2) If the rate of decrease of the radioiodide concentration in the blood is appreciable compared with the rate of equilibration of radioiodide between thyroid gland and blood, the ratio of the radioiodide concentrations in thyroid gland and blood will be larger than the corresponding ratio for freely exchangeable stable thyroid ioidide; 3) The thyroid clearance of iodide from the blood will not be a constant independent of the time after injection.

An analysis is also given of factors controlling the thyroid clearance, with criteria to determine which factors are rate controlling in special cases of importance.

REFERENCES

BROWNELL, G. L.: *J. Clin. Endocrinol.* **11**: 1095. 1951.

DOUGHERTY, J., J. GROSS AND C. P. LEBLOND: *Endocrinology* **48**: 700. 1951.

INGBAR, S. H.: Personal communication, 1952.

KETY, S. S.: *Pharmacol. Rev.* **3**: 1. 1951.

KETY, S. S.: *Methods in Medical Research* **1**: 204. 1948.

MAJOR, R. H., *Am. J. Anat.* **9**: 475. 1909.

MYANT, N. B., E. E. POCHIN AND E. A. G. GOLDIE: *Clin. Sc.* **8**: 109. 1949.

ODDIE, T. H.: *Brit. J. Radiol.* **22**: 261. 1949.

PITT-RIVERS, R.: *Physiol. Rev.* **30**: 194. 1950.

POCHIN, E. E.: *Lancet* **2**: 41. 84. 1950.

RIGGS, D. S.: *Pharmacol. Rev.* **4**: 284. 1952.

ROCHE, J., R. MICHEL, O. MICHEL AND S. LISSITZKY: *Biochim. et Biophys. Acta* **9**: 161. 1952.

SMITH, H. W.: The Kidney. New York, Oxford Univ. Press. 1951.

STANBURY, J. B. AND J. B. WYNGAARDEN: *Metabolism* **1**: 533. 1952.

STANLEY, M. M.: *J. Clin. Endocrinol.* **9**: 941. 1949.

STANLEY, M. M. AND E. B. ASTWOOD: *Endocrinology* **42**: 107. 1948.

TAUROG, A., W. TONG AND I. L. CHAIKOFF: *J. Biol. Chem.* **191**: 677. 1951.

WOLLMAN, S. H.: *J. Nat. Cancer Inst.* **13**: 807. 1953.

WOLLMAN, S. H. AND R. O. SCOW: *Endocrinology.* **53**: 332. 1953.

WOLLMAN, S. H., R. O. SCOW AND H. P. MORRIS: *J. Nat. Cancer Inst.* **14**: 593. 1953.

WOLLMAN, S. H. AND E. ZWILLING: *Endocrinology* **52**: 526. 1953.

METAHYPOPHYSEAL DIABETES PRODUCED BY GROWTH HORMONE[1]

JAMES CAMPBELL, L. CHAIKOF AND I. W. F. DAVIDSON
Department of Physiology, University of Toronto, Toronto, Canada

IT WAS found by Cotes, Reid and Young (1949) that purified growth hormone preparations induced idiohypophyseal, or temporary pituitary, diabetes in cats. This was shown also in dogs (Campbell, Davidson, Snair and Lei, 1950). Permanent pituitary or metahypophyseal diabetes has been produced by purified growth hormone in the partially depancreatized dog (Houssay and Anderson, 1949), and in dogs that had previously been given partially-purified diabetogenic preparations on one or more occasions (Campbell, Davidson and Lei, 1950; Campbell, Lei and Davidson, 1951). Metahypophyseal diabetes has now been produced in intact, previously untreated dogs by still further purified growth hormone. These results are reported together with observations on the characteristics of this diabetic state.

METHODS

The growth hormone preparations were dissolved (10 mg./ml.) in sterile, pyrogen-free saline and were injected subcutaneously with sterile syringe and needle. The skin was shaved, cleaned and painted with iodine prior to injection. Samples of venous blood were withdrawn in the morning, then injections were given, followed by the first meal. After 8 hours the second injection and the second meal were given. The prepared dog foods were *Chum*, Canada Packers Limited, a moist cooked ration composed of ground meat, grains or starches, minerals, etc., and compressed dry dog chow manufactured by the Ralston Purina Company (Table 3). The analytical methods have been described by Campbell, Hausler, Munroe and Davidson (1953).

EXPERIMENTAL

The growth hormone preparations that produced permanent diabetes were obtained by three methods. The preparations designated W were obtained by the method of Wilhelmi, Fishman and Russell (1948) from Fraction A. The dogs P and W received W5 and dogs B and J received W8. The other two growth hormone preparations, termed DP and DKP, were made by the methods of Campbell and Davidson, to be described. The dog M received the DP preparations, Nos. 20, 24 and 26, while dog T received the DKP preparations Nos. 21, 22, and 23 (Table 2).

Received for publication June 3, 1953.

[1] This investigation was aided by grants from the National Research Council and the National Cancer Institute of Canada.

TABLE 1. GROWTH-PROMOTING ACTIVITY

Assays of growth-promoting activity by the tibia test of Greenspan, Li, Simpson and Evans (1949). Female, Sprague-Dawley rats of 75 to 80 gm. body weight were hypophysectomized, and after 14 days the test preparations in 0.5 ml. saline were injected intraperitoneally for 4 days. The width of the epiphyseal cartilage of the tibia was measured 24 hours after the last injection.

Assay No.	Preparations injected		No. rats	Responses width of tibial disc		Comparative activity % of D19P
	No.	Dose, total in 4 days μg. per rat		Microns	Increase microns	
3.51	D20P	100	6	294	150	63
		40	6	254	110	
2.52	D24P	100	6	277	122	79
		40	6	259	104	
4.52	D26P	100	3	275	140	130
		40	6	237	101	
2.52	D21KP	100	6	315	159	100
		40	6	267	111	
4.52	D22KP	100	6	246	110	65
		40	6	210	75	
1.52	D23KP	100	6	388	154	73
		40	6	299	66	

The growth-promoting activity of W5 was tested by the body weight method of Marx, Simpson and Evans (1942) in groups of 7 young female hypophysectomized rats. Doses of 10, 25 and 100 μg. per rat per day for 10 days increased the body weight by 8, 14 and 15 gm., these responses indicating activity of about two-thirds that of the growth hor-

TABLE 2. METAHYPOPHYSEAL DIABETES, INDUCTION AND INSULIN REQUIREMENTS

The dogs were given Diet 3 during the induction period. Dogs P and W received insulin for 17 and 64 days respectively before pancreatectomy. The insulin requirements were determined on Diet 1, containing raw pancreas.

Dog	Induction period			Metahypophyseal diabetes				
	Growth hormone preparation	Average dosage per kg. per day mg.	Duration of injections days	Period without insulin days	Insulin requirement, units per day			
					Before pancreatectomy		After pancreatectomy	
					IZ*	PZI*	IZ	PZI
P	W5	3.03	27	44	6	4	12	6
W	W5	3.5	3	45	16	8	16	8
B	W8	3.5	26	4				
J	W8	3.5	36	52	8	4		
M	DP, Nos. 20, 24, 26	3.5	15	92	8	8		
T	DKP Nos. 21, 22, 23	3.5	37	150	6	6		

* IZ, insulin-zinc and PZI, protamine-zinc insulin of the Connaught Medical Research Laboratories.

TABLE 3. DIETS

These daily diets were given as two meals, 8 hours apart. Lean ground horse meat and compressed dog food ("Purina" dog checkers) were used

Diet No.	Foodstuffs, grams				Nutrients, calculated, grams			
	Pancreas	meat	chow	sucrose	Calories	protein	fat	carbohydrate
1	200	400		40	866	103	30	46
2		400	100	40	953	105	17	95
3		600	200	80	1680	172	26	190
4		300	300	40	1480	137	13	205
5	Canned dog food ("Chum")				640	56	17	65

mone preparation W4P described by Campbell *et al.* (1950). The growth-promoting activities of the other preparations were assayed by the tibia test of Greenspan, Li, Simpson and Evans (1949) in groups of 5 to 7 young female hypophysectomized rats given intraperitoneal injections of the preparations for 4 days, beginning 14 days after operation. In order to produce comparable results, a reference preparation of growth hormone, D19P, was given at 2 or more dosage levels to groups of hypophysectomized rats in each assay. Total doses of 40 and 100 μg. of W8 per rat increased the width of the proximal, uncalcified cartilaginous disc of the tibia by 73 and 117 microns, over the control value. This response is equivalent to about 50% of the standard preparation D19P. The growth responses to the DP and DKP preparations indicated that both possessed very high activity, about equal to that of D19P (Table 1). At the total dose of 40 μg. of these preparations the average increases in the width of the tibias were 105 and 84 microns respectively, while at the total dosage of 100 μg. the discs were increased in width by 137 and 141 microns respectively (Table 1). Direct comparison of the growth activities of these preparations with that of others is not possible at present due to lack of a standard reference preparation of growth hormone, so that comparisons must be made on the basis of biological response. The responses obtained by Greenspan *et al.* (1949) to the bovine growth hormone of Li, Evans and Simpson (1945) were 105 and 146 microns at these total doses of 40 and 100 μg. respectively. The responses obtained in the two laboratories to the different bovine growth hormone preparations are thus essentially the same, although it must be remembered that the conditions of the assay may have an influence on the results.

The adrenocorticotrophic activity was estimated by depletion of the ascorbic acid of the adrenal glands in hypophysectomized rats, according to Sayers, Sayers and Woodbury (1948). On the day after hypophysectomy the left adrenal gland was removed under amytal anaesthesia and the test substances in 1 ml. saline were given intravenously. After 1 hour the right adrenal gland was removed and the ascorbic acid content of each gland was determined. Growth preparation W5 in doses of 100, 50, 20 and 10 μg. to groups of 5 or 6 hypophysectomized rats decreased the average ascorbic acid concentrations of the right as compared to the left adrenal by 159 ± 8.8, 144 ± 11.2, 121 ± 11.0 and 39 ± 14.6 mg.% respectively. The standard errors are given. These results are directly comparable to those of Campbell, Davidson, Snair and Lei (1950, Table 3) and indicate that the growth hormone preparation W5 is about equivalent in ACTH activity to 2.8% of ACTH, S10 and to about 15% of ACTH, 41-L (Armour). The latter is said to contain about 68% of the ACTH activity of the ACTH standard, La-1.A.

The ACTH activity of the DP and DKP preparations was estimated to be low, since clear evidence that the doses given (100 to 1000 μg.) contained demonstrable amounts of ACTH was not obtained (Campbell and Davidson, to be published). Many more assays

would be required, however, to determine the actual level of possible contamination.

The thyroid-stimulating activity was measured by the method of Campbell, Gemeinhardt and Britton (1952), which is a modification of the method of Ghosh, Woodbury and Sayers (1951). Groups of hypophysectomized rats were given subcutaneous injections twice daily of the test preparations. The last of these injections was at 72 hours and at this time also I^{131}, without carrier, was given intraperitoneally. The animals were sacrificed 8 hours later and the radioactivity of the thyroid glands was determined. By this method the growth hormone preparation D20P was found to contain 0.1 U.S.P. units of thyrotropin per mg. No thyroid-stimulating activity was detected in the growth hormone preparations D21KP and D23KP.

Combined test for adrenocorticotrophin, thyrotrophin and gonadotrophins

The adrenal repair test of Simpson, Evans and Li (1943) was used to test for contaminants in D33KP. Total doses of 5 mg. in 4 days in young female hypophysectomized rats increased the body weight from 83 to 103 gm. The adrenal glands were increased slightly in weight (14.5 mg. in the test, and 12.3 mg. in the control groups) but not quite in proportion to body weight. No histological changes were noted. The cells of the thyroid acini and the general appearance of the glands were the same in both groups. No gonadal stimulation was observed. Thus these tests on the growth hormone obtained by the DKP procedure did not reveal, with the dosages and conditions employed, the presence of TSH or gonadotrophins.

The results of the electrophoretic and ultracentrifugal analyses, to be reported, indicate a high degree of homogeneity and correspondence with the findings of Li *et al.* (1945) and of Wilhelmi *et al.* (1948).

Induction of permanent diabetes

Permanent diabetes has been produced in six dogs given purified growth hormone. Some of the findings in three of these dogs (P, W and J) have been reported by Campbell, Davidson and Lei (1950) and by Campbell, Lei and Davidson (1951). Prior to the injections that resulted in permanent diabetes, two of the dogs (P and W) had been given purified growth hormone, which produced temporary diabetes, and before this had also been used to test the diabetogenic activities of other pituitary preparations. The dogs (B and J) had on a previous occasion been given six daily injections of a partially-purified anterior pituitary extract, in order to test them for sensitivity to the diabetogenic effects. The signs of diabetes elicited by the injections disappeared in two days. In all cases a month elapsed before giving the injections of purified growth hormone which led to permanent diabetes. Thus the blood sugar levels were normal and sugar was not present in the urine for some weeks before starting these injections. The dogs M and T had not been treated in any way prior to the series of injections of growth hormone that resulted in metahypophyseal diabetes.

During the period of induction of permanent diabetes by injection of the growth hormone, polyuria, polydipsia, hyperglycaemia, glycosuria and

ketonuria occurred. Towards the end of the injections the morning blood sugar was usually over 300 mg.% and about two litres of urine, containing over 100 gm. of sugar, were excreted daily. Towards the end of the injection period the tests for ketone bodies in the urine became especially strong. Eventually the dogs exhibited lassitude, refused food occasionally and vomited frequently. The dosage of the growth hormone preparations was from 3 to 3.5 mg. per kg. per day (Table 2). In 5 cases permanent diabetes was produced by 15 to 37 days of injection of growth hormone. In dog W, however only 3 injections were required, and insulin had to be administered for 2 days immediately following the last injection in order to rescue him from diabetic coma produced by growth hormone. It would seem probable that this dog had been made susceptible to the diabetogenic effects of the purified material by the previous periods of injection.

Permanent Pituitary Diabetes

One of the dogs (B) died from some unknown cause four days after the last injection of growth hormone, so that the permanence of the diabetes is not established in this case. Until death, the blood sugar was high and sugar was excreted in the urine. At autopsy, performed within 8 hours of death, long "goose-fat" plasma clots were found in the pulmonary and caval veins. These were apparently of post-mortem origin, as they were not obviously adherent to the walls of the vessels, and on histological examination did not exhibit lines of Zahn. Only a small amount of insulin was extractable from the pancreas (Table 5) and the beta cells of the pancreatic islets were few and poorly granulated. The other tissues appeared normal.

In the other five dogs the diabetes persisted throughout long periods of observation, without signs of diminution, after the injections of growth hormone were stopped. The periods of diabetes without insulin ranged from 44 to 150 days (Table 2) and the entire periods of observation, including the periods prior to and after giving insulin, for dogs P, W, J, M, and T were 80, 109, 404, 285 and 251 days. These periods were terminated by pancreatectomy in dogs P and W, while M and T are still under observation.

During the stage of metahypophyseal diabetes without insulin injections the urine volumes and the amounts of sugar excreted were remarkably high. Although the body weights did not exceed 15.5 kg. the average urine volumes during the whole of this period were 2800, 2600, 2600, 1100 and 780 ml. per day in dogs P, W, J, M and T respectively, while the corresponding average amounts of sugar excreted were 200, 200, 130, 85 and 52 gm. The concentrations of sugar in the urine ranged from averages of 5 to 7.9 %, the maximum observed on single days being about 10%.

In the dogs J on Diet 3, and T on Diet 5, less sugar was excreted than

the calculated amounts of total preformed carbohydrate in the diets. In dogs P, Diet 3, and W, Diets 4 and 5, the sugar excretion about equalled, or slightly exceeded the total carbohydrate ingested. In dogs P, Diet 2, and M, Diet 5, the average amounts of sugar in the urine exceeded the dietary carbohydrate by 30 and 17 gm. per day respectively. The above comparison may be questioned, however, since the availability of the dietary carbohydrate to the tissues was not determined. The sugar excretion may possibly be under-rated relative to the intake.

The nitrogen excretion of the permanently diabetic dog P on Diet 2, and without insulin, ranged from 8.1 to 16.0 gm., average 13.8 gm. per day for 7 days. The nitrogen intake on this diet was 16.8 gm. Since an average of 126 gm. of sugar was excreted, the proportion of gm. glucose per gm. nitrogen was 9.1. With a correction for the preformed carbohydrate of the diet, the D/N ratio was 126-95/13.8 = 2.2. It should be noted that this quotient was obtained on a mixed diet that contained both animal and vegetable protein.

These results cannot be compared to those of Marks and Young (1939) due to differences in diets. In an extensive study, these authors found that the sugar excretion of metahypophyseal diabetic dogs, on a diet of meat and without insulin, was greatly in excess of the small amount of carbohydrate in the food. Under these conditions it was found that the D/N ratio was high, ranging from 3.09 to 3.95.

The permanent diabetes produced by growth hormone in dogs was tolerated remarkably well. Nevertheless some deterioration in condition became apparent. The body weight declined, due in all probability to the losses of sugar and nitrogen in the urine and to the increasing acidosis. It was found, in agreement with the observations of Marks and Young, that this weight loss, and apparently some of the accompanying unfavourable effects on the animals, could be prevented to some extent by increasing the food intake. The effectiveness of this treatment was limited, however, for although dogs P, W and J received finally, in the metahypophyseal diabetic state without insulin, very rich diets (Nos. 3 and 4), the metabolic deficiencies were not overcome in the latter two dogs. In them, the turn to an unfavourable state appeared to coincide with digestive upset.

Ketones disappeared from the urine on ceasing the injections of growth hormone, but later appeared, and the qualitative test (Rothera's) became increasingly strong. Failure of appetite, vomiting and dehydration were noted eventually. It appeared that the administration of insulin was necessary, after 45 and 52 days of diabetes in dogs W and J respectively, in order to prevent collapse. The dogs P, M and T were still in relatively good condition, however, at the end of 44, 92 and 150 days of diabetes

TABLE 4. THE URINARY EXCRETION OF METAHYPOPHYSEAL DIABETIC DOGS, UNTREATED WITH INSULIN

Dog	Body weight, kg.	Period observed, days	Diet No.	Urine, average daily values, with ranges		
				Volume, ml. per kg. body weight	Sugar excretion, gm. per kg. body weight	Sugar concentration, gm. per 100 ml. urine
P	15.5	29	3	185 (146–210)	12.9 (10.5–15.4)	6.98 (5.91–7.91)
	15.2	15	2	105 (62–128)	8.3 (5.4–10.1)	7.80 (6.22–8.50)
W	9.5	25	4	265 (250–305)	21.7 (18.9–23.8)	7.77 (7.35–8.10)
		4	3	256 (237–284)	20.2 (18.1–21.4)	7.50 (7.30–7.65)
B	15.3	4	2	156	11.1	7.10
J	9.8	42	3	265 (61–388)	13.3 (6.5–19.4)	5.02 (4.0–6.1)
M	9.0	69	5	121 (11–290)	9.1 (3.5–24.8)	7.6 (2.4–10.9)
T	8.6	39	5	87 (24–210)	5.8 (1.6–13.6)	6.9 (1.9–9.8)

without insulin (Table 2). The dogs W, M and T lost weight during this period to the extents of 1.3, 3.5 and 2.9 kg. respectively, but the weights of P and J were not much altered.

The administration of insulin promptly reduced the hyperglycaemia, glycosuria, ketonuria and polyuria and caused a rapid gain in body weight. The improvement in appetite and in apparent general well-being on giving insulin was no less dramatic. When the diabetes was controlled so that 2 to 12 gm. of sugar were excreted with Diet 1, the insulin requirements of these permanently diabetic dogs were similar to those of depancreatized dogs (Table 2). Following pancreatectomy, the insulin requirement of dog W was unchanged, while that of dog P was apparently increased, although it is doubtful whether this difference is significant (Table 2).

The extractable insulin of the pancreas of these diabetic dogs was determined by the mouse-convulsion method (Wrenshall, 1951). In dogs P and W only about 0.5% of the normal amount was found (Table 5).

The results of the histological examinations of the pancreases will be reported by Dr. W. Stanley Hartroft. In general, the amount of islet tissue was reduced relative to the acinar tissue. In the islets of Langerhans fewer beta cells were present, relative to alpha cells, than in normal islets. The beta cells were very deficient in granular material, and were, in some

instances, vacuolated. The reduction in the amount of beta cell granulation agreed well with the amounts of insulin extractable from the pancreas. These findings are in agreement with those of Best, Campbell, Haist and Ham (1942) on the extractable insulin and histological changes of the pancreas in dogs with permanent pituitary diabetes.

It may be concluded that metahypophyseal diabetes was produced in these dogs given purified growth hormone.

DISCUSSION

The diabetes produced by these growth hormone preparations has persisted for periods of observation up to 400 days and since no signs of diminution in the intensity of the diabetes occurred, it appears that the diabetes is permanent. As is evident from Table 4, the diabetes is severe. It is remarkable that the dogs can live in this diabetic state for long periods, up to 150 days, without the administration of insulin. In comparison, depancreatized dogs given raw pancreas in the diet, can survive for relatively short periods (about 7 to 12 days) without insulin, although in them the concentrations of sugar in the blood and the amounts of sugar excreted are not greater. The presence of the pancreas in the dogs made diabetic by growth hormone evidently permits of this longer survival. Whether this can be attributed to a small residuum of islet cells secreting insulin, or to some other influence of the pancreas, is at present unknown.

When insulin is given to these diabetic dogs there is a marked improvement in alertness and activity, and weight is gained rapidly. The insulin requirements were not notably different from those of depancreatized animals, and pancreatectomy did not cause much change in the insulin requirements of two of the dogs. In some instances of dogs made perma-

TABLE 5. EXTRACTABLE INSULIN OF THE PANCREAS

The metahypophyseal diabetic dogs P and W were depancreatized 37 and 64 days respectively from the start of the insulin treatment. The dog J received injections of growth hormone for 4 days, total dose 12.5 mg. per kg. of body weight, with the usual dose of insulin and died in diabetic acidosis during the night, the pancreas being removed in the morning.

Metahypophyseal diabetic dogs	Body weight, kg.	Pancreas weight, gm. per kg. body wt.	Extractable insulin of the pancreas, units	
			Per gm. pancreas	Per kg. body wt.
P	13.1	2.65	0.021	0.070
W	12.6	1.44	0.022	0.031
B	15.3	3.36	0.071	0.24
J	10.7	1.57	(0.049)	(0.08)
Normal dogs,				
Means of 5	10.4	1.83	4.52	7.81
Range, from	8.5	1.42	2.41	5.90
to	13.7	2.75	6.44	11.2

nently diabetic by anterior pituitary extracts the insulin requirements are higher than those of depancreatized dogs, but in other instances the requirements are not different (Young, 1938; Marks and Young, 1939; Campbell and Best, 1938; Campbell, Keenan and Best, 1939).

Insulin can be extracted from the pancreases of the dogs made permanently diabetic by growth hormone but the amount is about 0.5 to 1.5 per cent of normal. Histological observations show loss of islet tissue with particularly severe destruction of the beta cells.

Thus the diabetes produced by purified growth hormone may be characterized by its permanence, great intensity, survival without insulin for long periods, improvement of condition produced by insulin, insulin requirements that were in the range of those expected of depancreatized dogs, the minor effects of pancreatectomy on the insulin requirements, the histological changes in the islets of Langerhans and the very low amounts of insulin extractable from the pancreas. These signs have been observed previously in the permanent pituitary or metahypophyseal diabetes produced in dogs by crude or partially purified anterior pituitary extracts (Young, 1937; Richardson and Young, 1938; Marks and Young, 1939; Campbell and Best, 1938; Campbell, Keenan and Best, 1938; Houssay, 1942; Haist, Campbell and Best, 1940). It may therefore be concluded that purified growth hormone alone can produce metahypophyseal diabetes.

From the results of the biological assays and other tests of the growth preparations, the thyroid-stimulating hormone, adrenocorticotrophic hormone, interstitial cell-stimulating hormone and the follicle-stimulating hormones can be ruled out as necessary participants in the result. While definite assays are not yet available, preliminary tests indicate that prolactin is not necessarily involved. The available data support the conclusion that the permanent diabetes was caused by the growth hormone of the anterior pituitary gland. The possibility that the diabetes was caused by an unidentified principle associated with the growth hormone cannot be excluded with certainty on chemical grounds. On the other hand, physiological experiments, described by Campbell, Munroe, Hausler and Davidson (1953), provide evidence for the deduction that the growth hormone may first stimulate the pancreatic islets to increased insulin-secreting activity, and that continued stimulation by excess of the growth hormone may lead to overwork of the beta cells and finally to their atrophic state characteristic of permanent pituitary diabetes.

It may be noted that the concentration of sugar in the urine of the metahypophyseal diabetic dogs, without insulin, reached a maximum of about 10 per cent. In view of the very large amounts of sugar excreted and the high urine volumes, it would appear probable that this may represent

approximately the maximum concentrating ability of the kidney tubules under these conditions.

SUMMARY

Permanent pituitary or metahypophyseal diabetes has been produced in dogs by purified bovine growth hormone. The thyroid-stimulating, adrenocorticotrophic, interstitial cell-stimulating and follicle-stimulating hormones are excluded as necessary participants in the production of the diabetes. Prolactin also does not appear to be involved.

The diabetes produced by growth hormone has persisted throughout the periods of observation, up to 400 days, without signs of diminution. The diabetes is intense, but can be tolerated for long periods, up to 150 days of abservation, without insulin. However, insulin improves the condition of the dogs. The insulin requirements were within the range of those found for depancreatized dogs. Pancreatectomy of the metahypophyseal dogs did not produce marked alteration in the insulin requirements. The acinar tissue of the pancreas is normal histologically, but the islet tissue is reduced and the beta cells show severe degeneration. The insulin extractable from the pancreas is very low.

Acknowledgements

We desire to express our gratitude to Professor C. H. Best for his interest and support. We are indebted to Dr. G. A. Wrenshall for determinations of the extractable insulin of the pancreas and to Dr. W. Stanley Hartroft for the histological examination of the tissues.

REFERENCES

Best, C. H., J. Campbell, R. E. Haist and A. W. Ham: *J. Physiol.* **101:** 17. 1942.
Campbell, J. and C. H. Best: *Lancet* **1:** 1444. 1938.
Campbell, J., I. W. F. Davidson and H. P. Lei: *Endocrinology* **46:** 588. 1950.
Campbell, J., I. W. F. Davidson, W. D. Snair and H. P. Lei: *Endocrinology* **46:** 273. 1950.
Campbell, J., H. K. Gemeinhardt and Amy Britton: *Can. Physiol. Soc. Proc.* 1952.
Campbell, J., H. R. Hausler, J. S. Munroe and I. W. F. Davidson: *Endocrinology* **53:** 134. 1953.
Campbell, J., H. C. Kennan and C. H. Best: *Am. J. Physiol.* **126:** 455 p. 1939.
Campbell, J., H. P. Lei and I. W. F. Davidson: *Endocrinology* **49:** 635. 1951.
Campbell, J., J. S. Munroe, H. R. Hausler and I. W. F. Davidson: *Endocrinology* **53:** 549. 1953.
Cotes, P. M., E. Reid and F. G. Young: *Nature* **164:** 209. 1949.
Ghosh, B. N., D. M. Woodbury and G. Sayers: *Endocrinology* **48:** 631. 1951.
Greenspan, F. S., C. H. Lei, Miriam E. Simpson and H. M. Evans: *Endocrinology* **45:** 455. 1949.
Haist, R. E., J. Campbell and C. H. Best: *New Eng. J. Med.* **223:** 607. 1940.
Houssay, B. A.: *Endocrinology* **30:** 844. 1942.
Houssay, B. A. and Evelyn Anderson: *Endocrinology* **45:** 627. 1949.

LI, C. H., H. M. EVANS AND MIRIAM E. SIMPSON: *J. Biol. Chem.* **159**: 353. 1945.
MARKS, H. P. AND F. G. YOUNG: *J. Endocrinology* **1**: 470. 1939.
MARX, W., MIRIAM E. SIMPSON AND H. M. EVANS: *Endocrinology* **30**: 1. 1942.
RICHARDSON, K. C. AND F. G. YOUNG: *Lancet* **1**: 1908. 1938.
SAYERS, M. A., G. SAYERS AND L. A. WOODBURY: *Endocrinology* **42**: 379. 1948.
SIMPSON, MIRIAM E., H. M. EVANS AND C. H. LI: *Endocrinology* **33**: 261. 1943.
WILHELMI, A. E., J. B. FISHMAN AND JANE A. RUSSELL: *J. Biol. Chem.* **176**: 735. 1948.
WRENSHALL, G. A.: "Studies on the Extractable Insulin of the Human Pancreas." Ph.D. Thesis, University of Toronto, 1951.
YOUNG, F. G.: *Lancet* **2**: 372. 1937.
YOUNG, F. G.: *Brit. Med. J.* **ii**: 393. 1939.

THE ACTION OF RELAXIN ON MAMMARY GLAND GROWTH IN THE RAT[1]

THOMAS C. SMITH[2,3]

The Biological Laboratories, Harvard University, Cambridge, Massachusetts

SMALL doses of progesterone injected simultaneously with estrogens produce optimal lobulo-alveolar growth in the mammary glands in the mouse (Mixner and Turner, 1940) or rabbit (Lyons and McGinty, 1941), while in the rat relatively larger doses of progesterone are required to elicit a comparable reaction (Curtiss, 1949). Hamolsky and Sparrow (1943) first showed that relaxin, injected simultaneously with small doses of estradiol and progesterone, resulted in good lobulo-alveolar growth in immature, estradiol-primed, ovariectomized rats. Administration of a combination of any two of these hormones in the dosage used yields negative results. There was little or no mammary response to any combination in the ovariectomized, hypophysectomized rat. This paper is a further investigation into the effects of relaxin on the mammary glands of the rat.

MATERIAL AND METHODS

Relaxin made up in normal saline was injected subcutaneously twice daily. The steroids were also injected subcutaneously every day using a concentration of 20 μg. of estradiol per ml. of oil and 20 mg. of progesterone per ml. of oil.[4] Growth hormone, 100 μg. daily, ACTH, 1.0 mg. daily, and prolactin, 2.0 mg. daily were injected in saline.[5]

Rats of the Harvard colony, fed a basic diet of Rockland pellets *ad libitum*, were ovariectomized at twenty-four days of age. Injections of 1.0 μg. estradiol daily were begun four days later, and continued for twelve days. Some rats were also thyroidectomized at the time of ovariectomy, while others were hypophysectomized or adrenalectomized at the end of

Received for publication June 13, 1953.

[1] Supported in part by a grant to Prof. Frederick L. Hisaw from the United States Public Health Service.

[2] A portion of this work was carried out as an Atomic Energy Commission Predoctoral Fellow in the biological sciences, administered by the National Research Council.

[3] Present address: Boston University, School of Medicine, Department of Pharmacology.

[4] The steroids were generously supplied by the Schering Corporation, Bloomfield, New Jersey.

[5] Pituitary preparations: Growth Hormone, Armour, 22-KR2; ACTH, Armour, 71-50-R; Prolactin, Schering ST 45, 24 I.U. per mg.

the estrogen priming period. Hypophysectomized rats were fed 5% glucose in their drinking water; adrenalectomized rats were maintained on 1% glucose and 1% NaCl in their drinking water. All groups were further subdivided and injected with 1.0μg. estradiol, 1.0 mg. progesterone and relaxin, alone and in various combinations for 7, 12, or 18 days.

Two sets of experiments on mature rats were also undertaken in order to compare their responses to those of immature rats, and to study the effects of the length of castration on response to the steroids and relaxin. In the first series, animals were ovariectomized at 24 days of age, and used at four months of age or castrated at 4 months of age and used immediately. Both groups were pretreated with estrogen, and one animal in each group was killed at the end of pretreatment. The remaining animals were continued on the same combination of hormones as for the immature rats. In the second series of experiments animals were ovariectomized at three months of age, and injected either with estrogen alone or with estrogen and higher doses of progesterone (3 to 5 mg. daily) or with the combination of estrogen (1 μg.), progesterone (1 mg.) and relaxin (180 to 270 G.P.U.) daily. The response of the mammary glands of these older animals was compared with that of immature rats treated similarly.

Animals were killed by a blow on the head, the abdominal-inguinal mammary glands were removed, spread in a Petri dish and fixed for two to three hours in Bouin's fluid. The area of the glands could then be clearly visualized under a dissecting microscope and traced onto thin sheets of paper affixed to the back of the dish. Areas were determined from these tracings with a planimeter. The glands were weighed on a triple-beam balance after trimming off the peripheral fat and connective tissue. Details of mammary gland structure were studied from both whole mounts and sections. Lobulo-alveolar response of the mammary glands was rated for each whole mount from 1 to 4 on an arbitrary scale, and the average degree of response for a group was calculated as the average of the sum of individual responses. The percentage or number of animals showing positive responses was also recorded, counting a borderline response as one half.

Histochemical tests were also run on the mammary tissues. Alkaline phosphatases were determined by the method of Gomori (1939). Chemical estimations of the enzyme were made on water extracts of weighed portions of mammary gland, using the method of King and Armstrong (1934). Nucleic acids were localized by methyl-green, pyronin staining and by the Felugen reaction. The basophilia due to ribonucleic acid was confirmed by pretreatment of the sections with ribonculease or by extraction with 10% perchloric acid in the cold. Metachromatic reactions were tested by toluidin blue staining after fixation in basic lead acetate-formalin. The presence of reticulin and collagen was demonstrated by ammoniacal silver impregna-

tion of the reticulin followed by counterstaining with Mallory's triple stain after fixation of the tissue in Rossman's fluid. Lipids were colored with Sudan black B after fixation in formol-calcium.

Some of the rats injected with the steroids and relaxin were not killed, but were used in a study of secretory changes. The day following the last injections of the sex hormones, a biopsy of mammary tissue was obtained by making a small incision in the skin just medial to the ventral periphery of the abdominal-inguinal glands, and removing a small wedge of the mammary tissue for fixation. After the first biopsy was taken, a single injection of prolactin (2 mg.) was made subcutaneously into the back of the neck and subsequent biopsies of the same gland were performed every day for four days. The amount of lipid colored by Sudan black was used as an indication of secretion.

RESULTS

Several crude and purified extracts of relaxin induced lobulo-alveolar growth in rats simultaneously receiving 1.0 μg. estradiol and 1.0 mg. progesterone daily. The steroid combination without relaxin induced little or no lobulo-alveolar growth in these dosages. The length of treatment with the steroids and relaxin had no influence on the percentage of animals showing positive lobulo-alveolar responses. However, the greatest degree of lobulation was attained after twelve or eighteen days treatment, and, although individual responses varied from one to four, the group response was approximately the same in different experiments whether crude or pruified extracts were employed. One relaxin preparation may have also aided in the promotion of ductal growth since several glands showed strikingly greater areas and very large club-shaped end buds, in addition to the lobulation.

Injection of relaxin concentrated from pregnant rabbit serum (PRS)[6] was also effective. The response was variable but positive in four rats. The concentrate contained only 0.5 G.P.U. per mg., making it necesssary to inject 50 mg. of material daily in order to deliver a dose of 25 G.P.U. There is no doubt that many contaminants were present in this crude preparation. However, prolactin was probably not present in appreciable quantities since, histologically, there was no evidence of secretion or of distension of the alveolar lumina in the mammary glands.

The action of one of the crude relaxin preparations was studied in further detail. Preparation P-46-2, 15 G.P.U./mg., was injected with 1.0 μg. estradiol or 1.0 mg. progesterone alone, or with the combination of the two steroids for twelve days (Table 1). Estradiol alone or estradiol and pro-

[6] Prepared by the method of Albert and Money (1946).

TABLE 1. ACTION OF CRUDE RELAXIN[1] IN COMBINATION WITH STEROIDS ON MAMMARY GROWTH

Group—daily treatment	No. of rats	Average body weight (gm.)		Area ($cm.^2$)	Mammary gland lobulation	
		Gain	At autopsy		Number of positive responses	Average degree per group
Estradiol, 1.0 μg.	3	27	132	6.5±0.61	1	0.3
Estradiol, 1.0 μg. Relaxin, 6 mg.	2	29	135	8.2	2	2.0–3.0
Estradiol, 1.0 μg. Progesterone, 1.0 mg.	2	20	128	6.5	0	—
Progesterone, 1.0 mg. Relaxin, 6 mg.	2	41	142	7.2	1	0.5
Estradiol, 1.0 μg. Progesterone, 1.0 mg. Relaxin:						
3 mg.	4	26	144	5.7±0.48	4	2.5
6 mg.	4	27	145	6.2±0.29	4	1.8–2.5
12 mg.	4	30	146	7.1±0.83	3	2.0–2.75
Uninjected Controls	3	50	170	4.3±0.21	0	—

[1] A saline extract of whole ovary residue assaying 15 G.P.U./mg.

gesterone gave only negligible lobulo-alveolar development. Furthermore, it should be noted that estradiol and relaxin elicited good lobulation, while progesterone and relaxin were only slightly effective. All doses of relaxin, injected simultaneously with estradiol and progesterone, led to good alveolar growth in thirteen of fourteen rats. The degree of lobulation was roughly comparable to that seen during mid-pregancy. There was also some indication that the addition of relaxin may have led to an increase in area of the mammary gland, indicative of ductal growth, but the differences were not striking.

Two highly purified preparations also elicited mammary lobulo-alveolar growth when injected with either estradiol or estradiol and progesterone (Table 2, Plate I). There was no difference in mammary area between groups treated with relaxin and the steroids and those receiving the steroids alone. Even though one rat in group 5 was sick at the termination of the experiment and had lost weight, its mammary glands showed excellent lobulation. In these experiments, as in others, the relaxin preparation did not influence the depression in body weight always seen during treatment with estrogen and progesterone.

Thus, either crude or purified extracts of relaxin elicited comparable lobulo-alveolar growth, but showed no consistent effect on ductal growth when given with non-lobulating doses of estradiol and progesterone. However, neither this combination of steroids and relaxin, nor com-

bination of estradiol and progesterone (5 mg. daily) sufficient to induce lobulation in the castrated rat, caused mammary growth in rats hypophysectomized after the estrogen-priming period. After twelve days' treatment the glands were found to have undergone atrophy and were reduced to thin, bare ducts. If only a small fragment of the hypophysis remained, good duct and alveolar growth usually ensued. The concurrent injection of prolactin or of growth hormone following hypophysectomy often led to some restoration of duct and alveolar response to the ovarian hormones and relaxin. However, this was not true for the adrenocorticotropic hormone.

In order to test whether hypophysectomy might produce some of its effects through inanition, a group of normal rats injected with the same combinations of hormones were put on a restricted food intake of 3 gm. per rat daily. The mammary glands of animals subjected to such treatment exhibited an entirely different appearance from those of hypophysectomized rats. Although the mammary area was strikingly reduced, lobulo-alveolar growth in response to the ovarian hormones and relaxin was unaffected by this acute regimen, which led to the death of 50% of the animals.

Thyroidectomized rats injected with estradiol, progesterone, and relaxin showed mammary glands with considerably greater lobulo-alveolar growth and slightly greater mammary areas than in the non-thyroidectomized controls treated with the same combination of hormones. However, the increase in lobulo-alveolar growth after thyroidectomy is not specifically related to relaxin action, since thyroidectomized rats injected with estradiol and progesterone, or even with the estrogen alone, had mammary glands with nearly maximal lobulation. Thus, the differences in lobulation be-

TABLE 2. COMPARISON OF THE ACTION OF PURIFIED PREPARATIONS OF RELAXIN ON MAMMARY GLAND GROWTH

Group—daily treatment	No. of rats	Average body weight (gm.)		Mammary gland			
		Gain	At autopsy	Weight (gm.)	Area ($cm.^2$)	Lobulation	
						%	Average degree
1) Estradiol, 1.0 μg.	3	30± 1.7	138± 2.6	1.07± .069	8.1±0.22	50	1
2) Estradiol, 1.0 μg. CH 31–95, 0.67 ml.[1]	3	32± 1.5	141± 2.7	0.92± .038	9.1±0.72	100	2-3
3) Estradiol, 1.0 μg. Rx-S-1, 20 mg.[2]	3	33± 3.5	135± 7.1	0.78± .090	8.6±1.35	100	2-3
4) Estradiol, 1.0 μg. Progesterone, 1.0 mg.	3	30± 0.4	143± 3.9	0.78± .018	8.6±0.26	50	1
5) Estradiol, 1.0 μg. Progesterone, 1.0 mg. CH 31–95, 0.67 ml.	3	19±11.5	133±12.2	0.75± .096	9.2±0.19	67	2-3
6) Estradiol, 1.0 μg. Progesterone, 1.0 mg. Rx-S-1, 2.0 mg.	3			0.85± .058	8.6±0.19	100	2

[1] CH 31–95, obtained from the Chilcott Laboratories, and containing 300 G.P.U./ml. by our assay.
[2] Rx-S-1, prepared from pregnant sow ovaries by the saline extraction-ethanol fractionation method of Frieden and Hisaw (1950), assaying 50 G.P.U./mg.

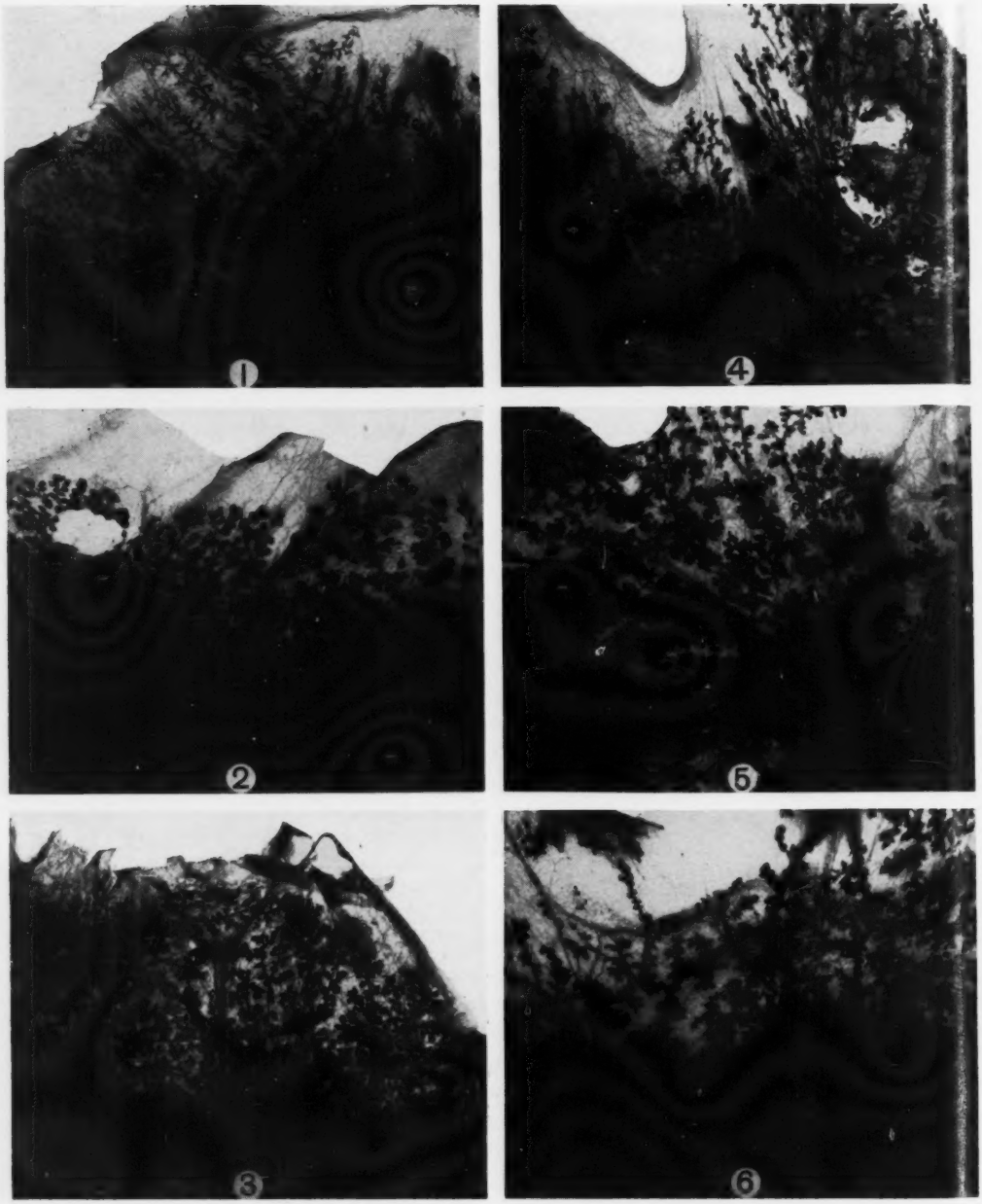


PLATE I. The Action of Relaxin-containing Extracts in Mammary Gland Growth.

tween mammary glands of castrated rats treated with the combination of estradiol, progesterone, and relaxin, or with the steroids alone, was no longer apparent in the castrated, thyroidectomized rat.

On the other hand, adrenalectomized rats injected with estradiol, progesterone, and relaxin had mammary glands which showed unaltered lobulo-alveolar growth, but were reduced in both area and weight. Average body weight was also considerably decreased in these animals, but the injection of DCA (0.5 mg. daily) restored normal rate of body growth and the ability of the mammary glands to respond to treatment with the ovarian hormones and relaxin, as in the castrated animal with intact adrenals.

Duration of castration had a slight effect on mammary gland response of the mature rat to estrogen priming and no effect on response to the combination of estradiol, progesterone, and relaxin. The response after seven or twelve days treatment was less in the mature than in the immature rat, even though the daily dose of relaxin was increased to 270 G.P.U. (Table 6). After eighteen days treatment, however, the response of the mammary glands of the mature rat became equal to that of the immature rat. The response of the older rat was similar to that of the immature animal, in that there was no lobulo-alveolar growth with 1.0 μg. estradiol and 1.0 mg. progesterone daily, but positive responses were seen when the dose of progesterone was raised to 3 to 5 gm. daily.

Mammary glands with comparable development showed similar histochemical patterns whether the growth was induced by estradiol, progesterone and relaxin or by estradiol and progesterone without relaxin. Alkaline phosphatase reactions were limited almost entirely to the parenchymal elements. Variations in degree of the reaction could not be correlated with either the nature or intensity of hormonal stimulation. Chemically, total activity was increased 2 or 3-fold in glands showing alveolar growth, whether induced by pregnancy, or by the steroid-relaxin combinations.

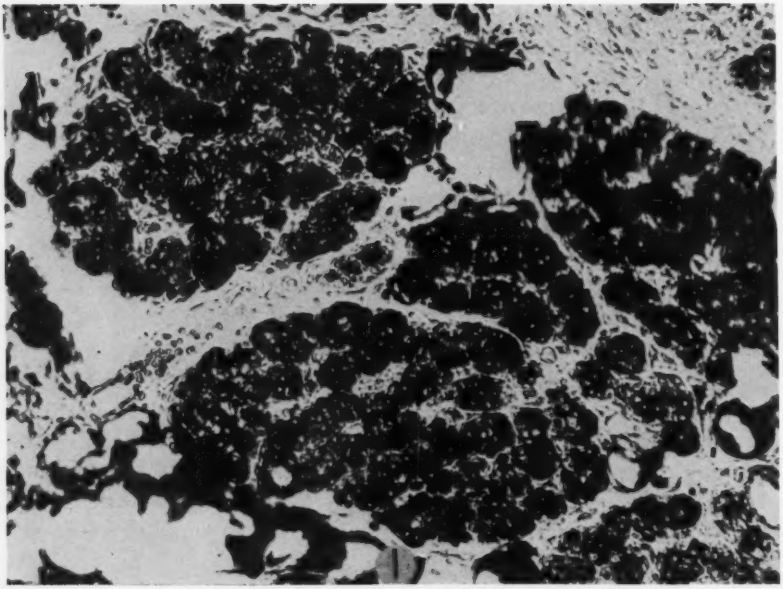
The cytoplasm of ducts and alveolar cells when stained with a modified Unna-Pappenheim mixture, showed pyronin-staining materials, which were

---

EXPLANATION OF PLATE I

Rats were ovariectomized at twenty-four days of age, allowed three days postoperative recovery, and injected with 1.0 μg. estradiol daily for twelve days. They were then treated with the following daily dosages for another twelve days, and autopsied the day following the last injections. All figures ×4.5.

FIG. 1. 1.0 μg. estradiol.
FIG. 2. 1.0 μg. estradiol and 200 G.P.U. CH 31–95.
FIG. 3. 1.0 μg. estradiol and 100 G.P.U. Rx-Sl.
FIG. 4. 1.0 μg. estradiol and 1.0 mg. progesterone.
FIG. 5. 1.0 μg. estradiol, 1.0 mg. progesterone, and 200 G.P.U. CH 31–95.
FIG. 6. 1.0 μg. estradiol, 1.0 mg. progesterone, and 100 G.P.U. Rx-Sl.



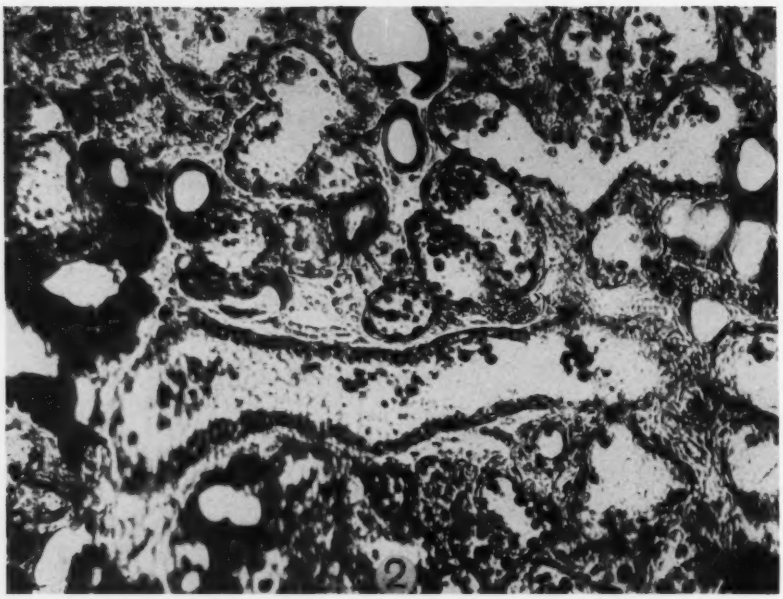


PLATE II. The Induction of Secretory Changes in the Experimentally-developed Mammary Gland.

TABLE 3. THE INFLUENCE OF AGE ON MAMMARY GLAND REACTIVITY TO RELAXIN

Daily dose of relaxin	Alveolar response of immature rat			Alveolar response of mature rat		
	Number of rats	Number responding	Average degree	Number of rats	Number responding	Average degree
7 days of injections						
90 G.P.U.	2	2	1.5	2	0	—
12 days of injections						
45 G.P.U.	4	4	2.5			
90	4	4	1.75–2.5	2	0	—
180	3	2.5	2.0 –2.75	3	2	1.6–2.0
270				3	2	1.3–2.0
18 days of injections						
90 G.P.U.	4	3	2.5	2	2	2.5–3.5

granular in appearance and extractable with cold 10% perchloric acid or 1% ribonuclease. Methyl green stained only a fine network in the nucleus, interspersed with pyronin-reacting substances, and the nucleoli stood out as bright rose-colored granules.

Metachromatic reactions for the ground substance, or argyrophilic reactions for reticulin, followed by counterstaining with Mallory's triple stain, did not reveal any striking differences between connective tissue of mammary glands from animals receiving estrogen, progesterone, and relaxin and those receiving the steroids alone.

After hormonal treatment mamary glands showed heavy concentrations of sudanophilic material in the alveolar epithelium and the amount of lipid was related to the degree of lobulo-alveolar development (Plate II, Fig. 1). Rats treated with estradiol alone had mammary glands composed mainly of ducts and buds, and these epithelia were relatively devoid of sudanophilic material. Glands of rats trested with combinations of estradiol, progesterone, and relaxin or with estradiol and progesterone effective in producing lobulo-alveolar growth were alike with respect to the sudanophilic reaction and also in their response to prolactin. Twenty-four hours after a

EXPLANATION OF PLATE II

Rat ovariectomized, treated with 1.0 μg. estradiol for twelve days, then with a combination of estradiol, progesterone, and relaxin for another twelve days.

FIG. 1. Section of biopsy 1, taken 12 hours after the last injection of relaxin. The epithelial cells of the alveoli are packed with lipid droplets. One subcutaneous injection of prolactin was made at this time. ×170.

FIG. 2. Section of biopsy 2, taken 24 hours after the injection of prolactin. The alveolar lumina are distended with secretory droplets, the epithelial cells are flattened and partially depleted of lipid. ×170.

single injection of prolactin, the alveoli showed evidences of secretion characterized by depletion and coalescence of sudanophilic material in the epithelium, droplets protruding into the lumina, and distention of the lumina with secretory products. At 48 hours, epithelial depletion had progressed, and fewer secretory droplets were present in the limina which were already contracting in size, and by 72 hours, the epithelium was depleted and the lumina were small and devoid of secretory material.

In rats injected with the ovarian hormones and relaxin, but given no prolactin, a progressive decrease in lipid of the alveolar epithelium occurred without any coalescence, secretion, or distention of the lumina. Similarly, rats treated with the same combinations of ovarian hormones, then adrenalectomized at the time of the initial biopsy and injected with prolaction, showed a progressive depletion of sudanophilic material in the alveolar epithelium without evidence of secretion.

Ductal and end-bud epithelia showed no change from an initial light coloring of sudanophilic material after injection of prolactin. Although a few droplets were present in the ductal lumina, these were probably transported from the alveoli and there was no evidence of secretion from the ducts themselves.

DISCUSSION

Estradiol alone or combinations of estradiol and progesterone which do not produce lobulation of the mammary glands of ovariectomized rats, will do so when ovarian extracts containing relaxin are added to the treatment. However, the negative response to progesterone alone is not modified by relaxin. The degree of lobulo-alveolar development in rats treated with combinations of estradiol, progesterone, and relaxin, though variable, is comparable to that seen between the tenth and twentieth day of pregnancy, and the growth elicited by these combinations was indistinguishable histologically and histochemically from that obtained with the estrogen and a higher dose of progesterone without the relaxin.

Whether the lobulating activity of these extracts is due to relaxin or some other factor in the preparation has not been answered conclusively by these experiments. Nevertheless, the ability of either a purified or crude preparation to induce a lobular response is related to relaxin content, suggesting that it is the responsible agent.

The reason that the mammary glands of mature rats were less responsive to the action of relaxin than those of immature rats is obscure. This may be related to the action of relaxin on the connective tissue elements, although no histochemical differences could be detected between the connective tissue stroma of mammary glands of immature and mature experimental animals.

It does not appear that alkaline phosphatase exerts a limiting or con-

trolling action in the growth of the parenchymal tissue. Certainly there was no parallelism between phosphatase concentration in individual cells and growth of these elements. The total amount of enzyme present, as shown by chemical assay, did increase with the amount of parenchymal tissue formed, and is therefore a fairly accurate index of the amount of that tissue in the gland.

Hypophysectomized rats given estradiol, progesterone, and relaxin show no ductal or alveolar development of the mammary glands. These findings are in agreement with those of Reece *et al.* (1936) and of Leonard and Reece (1942) who used only the steroids. However, when the steroids were supplemented with pituitary growth hormone or prolactin in our experiments, the response of the mammary glands was similar to that of non-hypophysectomized animals. It is also of interest that adrenocorticotrophic hormone is not able to restore the responsiveness of the mammary glands of the hypophysectomized animal.

Retardation of body growth as a consequence of hypophysectomy does not seem to be responsible for the failure of mammary gland growth under these conditions. When the ovarian hormones and relaxin are given to young castrated rats whose normal body growth has been prevented by restricting the diet only ductal growth is inhibited. This indicates that the effects of hypophysectomy on the response of the mammary gland are due to the removal of specific pituitary hormones.

Neither adrenalectomy nor thyroidectomy interferes with the growth of the alveolar elements induced by steroid-relaxin combinations. In fact, the presence of the thyroid seems to exert an inhibitory action on lobulo-alveolar growth, a phenomenon first noted by Leonard and Reece (1941). When this influence is removed doses of estrogen, or of estrogen and progesterone, which elicit little or no lobulation in rats with intact thyroids, produce marked lobulation.

Alveoli of the mammary gland developed by estradiol, progesterone, and relaxin are capable of secretion after the injection of prolactin as revealed by staining of lipid droplets. However, no spontaneous secretion was observed after the termination of sex hormone injections, and glands in which only ductal development occurs do not show evidences of secretion. Therefore, ductal secretion, if it occurs at all in the rat, must be a minor component of lactational secretion in this species.

Relaxin also has been shown to influence mammary growth in other species of laboratory animals. Trentin (1951) has shown that relaxin may slightly increase the percentage of mice showing lobulo-alveolar responses to simultaneously administered estrogen and progesterone. Likewise, Garrett and Talmage (1952) have found that it "potentiates" the action of estrogen on the alveolar system of the guinea pig, and causes increased arborescence of the mammary duct system of the rabbit. These reports and the results

of the present experiments show that relaxin preparations can influence lobulo-alveolar growth of the mammary gland in several species of laboratory animals, but there is insufficient evidence at present to conclude that they may also affect ductal growth.

SUMMARY

Relaxin extracts given in combination with estradiol or a combination of estradiol and progesterone which did not elicit any lobulo-alveolar growth by itself, caused marked lobulo-alveolar growth of the mammary gland of the immature, ovariectomized rat. Purified preparations gave as good responses as crude preparations, and glands showed an identical appearance histologically and histochemically with glands developed by estradiol and a higher dose of progesterone without relaxin. The mammary glands of mature rats were considerably less responsive to relaxin than those of immature rats.

Mammary glands of hypophysectomized rats showed negative ductal and lobular responses to relaxin-steroid combinations, while those of adrenalectomized rats showed only a reduction in total area. Mammary gland area of adrenalectomized rats was restored by simultaneous injection of DCA, in doses which produced no mammary growth alone. An intensely positive response was noted in thyroidectomized rats injected with relaxin, progesterone, and estradiol. However, this effect can be accounted for almost entirely by an increased response to estrogen alone.

The alveolar structures developed by steroid-relaxin combinations show evidences of secretion after a single injection of prolactin. There was no evidence of spontaneous secretion after the cessation of sex hormone injection, or of ductal secretion. Adrenalectomy interfered with the response to prolactin.

REFERENCES

ALBERT, A. AND W. L. MONEY: *Endocrinology* **38**: 56. 1946.
CURTISS, C.: *Endocrinology* **45**: 284. 1949.
FRIEDEN, E. H. AND F. L. HISAW: *Arch. Biochem.* **29**: 166. 1950.
GARRETT, F. A. AND R. V. TALMAGE: *J. Endocrinol.* **8**: 336. 1952.
GOMORI, G.: *Proc. Soc. Exper. Biol. & Med.* **42**: 23. 1939.
HAMOLSKY, M. AND R. C. SPARROW: *Proc. Soc. Exper. Biol. & Med.* **60**: 8. 1945.
KING, E. J. AND A. R. ARMSTRONG: *Canad. M.A.J.* **31**: 376. 1934.
LEONARD, S. L. AND R. P. REECE: *Endocrinology* **28**: 65. 1941.
LEONARD, S. L. AND R. P. REECE: *Endocrinology* **30**: 32. 1942.
LYONS, W. R. AND D. A. MCGINTY: *Proc. Soc. Exper. Biol. & Med.* **48**: 83. 1941.
MIXNER, J. P. AND C. W. TURNER: *Endocrinology* **30**: 591. 1940.
REECE, R. P., C. W. TURNER AND R. T. HILL: *Proc. Soc. Exper. Biol. & Med.* **34**: 204. 1936.
TRENTIN, J. J.: *Proc. Soc. Exper. Biol. & Med.* **78**: 9. 1951.

A CRITICAL ANALYSIS OF THE EOSINOPHILE RESPONSE IN RATS TO ACTH AND CORTISONE[1]

MARY E. DUMM AND ELAINE P. RALLI

Department of Medicine, New York University College of Medicine and New York University-Bellevue Medical Center, New York City, New York

A SUPPRESSION of the typical lymphopenic response to swimming and to ACTH in rats on pantothenate deficient diets has been reported from this laboratory (Dumm *et al.*, 1949; Ralli, 1950; Dumm and Ralli, 1953). The observations have been confirmed by Winters *et al.* (1952) who found that both the eosinophile and lymphocyte responses to ACTH and to epinephrine were diminished in the pantothenate deficient rat. They also observed that the lymphocyte and eosinophile counts responded normally to the injection of cortisone in the deficient rat.

The present report is concerned with the effects of ACTH and cortisone on the eosinophile counts of intact and adrenalectomized rats fed large amounts of pantothenate following a period of deficiency.

PROCEDURE

Experimental Animals

Young rats of the Long-Evans strain, 30 to 35 days of age, were placed on a diet deficient in pantothenic acid, but otherwise adequate, for 30 days and were then adrenalectomized. One group of rats was continued on the pantothenate deficient diet after adrenalectomy. In the other groups the diet was supplemented with 40 mg. of pantothenate per 100 gm. of diet. The rats consumed 10 to 15 gm. of the diet daily. Intact rats were subjected to the same dietary regime. As controls, a group of rats of the same age, never subjected to any dietary deficiency and kept on an adequate diet, were also studied before and after adrenalectomy.

The response of the eosinophiles to the injection of ACTH or cortisone was studied 4 days after adrenalectomy in the rats continued on the pantothenate deficient diet, and 7 and 20 days after adrenalectomy in rats transferred to the high pantothenate diet. All rats received 1% NaCl as drinking water. The completeness of adrenalectomy was established by withdrawal of salt. Results obtained on any animal which survived salt withdrawal were excluded.

Received for publication June 29, 1953.

[1] This research was aided by a grant from the Josiah Macy Jr. Foundation.

Blood Counts

The eosinophiles were counted before and 2, 4 and 6 hours after the injection of either ACTH or cortisone. Freely flowing tail blood was used for the blood samples. The eosinophiles were stained by the procedure recommended by Speirs and Meyer (1949) and counted by a direct chamber method.

Injected Material

A preparation of ACTH[2] with a potency of 1.6 times the Armour LA-1-A standard was dissolved at a concentration of 4 units/ml. in 0.01 M acetic acid, giving a solution of pH 4. The rats were injected intraperitoneally with 2 units of ACTH per 100 gm. rat weight.

The cortisone[3] was given in an aqueous suspension containing 4 mg. of cortisone acetate per ml. The rats were injected intraperitoneally with 2 mg. of cortisone acetate per 100 gm. of rat weight.

RESULTS

Effect of Adrenalectomy on Initial Eosinophile Counts

The initial eosinophile counts of intact and adrenalectomized rats on the various diets studied are summarized in Tables 1 and 2. It is apparent, both from the range of values and from the magnitude of the standard deviations, that the eosinophile levels in both intact and adrenalectomized rats varied widely. This has been reported by other observers (Hills *et al.*, 1948; Recant *et al.*, 1950; Best *et al.*, 1952; Tabor and Klopp, 1953). The discrepancy between the mean and the median eosinophile counts, which is seen in many of the groups, suggests that the values are not normally dis-

TABLE 1. INFLUENCE OF DIET ON THE RESTING LEVEL OF CIRCULATING EOSINOPHILES IN INTACT RATS

Diet history		Number of rats	Eosinophiles per cmm.			
Days on deficient diet	Days on high pantothenate diet		Median	Mean	S.D.	Range
(Control diet)		55	300	347	303	39–1191
35	0	16	232	457	478	14–1643
30	7	18	121	186	178	48–720
30	20	12	161	195	162	30–665
0	20	5	179	197	—	102–259

[2] We are indebted to the Armour Laboratories for the ACTH used in these experiments.

[3] We are indebted to the Merck Laboratories for the cortisone acetate used in these experiments.

TABLE 2. INFLUENCE OF DIET ON THE RESTING LEVEL OF CIRCULATING EOSINOPHILES IN ADRENALECTOMIZED RATS

Diet history		Days after adrenalectomy	Number of rats	Eosinophiles per cmm.			
Days on deficient diet	Days on high pantothenate diet			Median	Mean	S.D.	Range
(Control diet)		5–17	24	314	344	235	74–940
(Control diet)		10–17	19	548	628	310	120–1182
35	0	4	20	178	180	125	41–523
30	7	7	17	262	454	441	27–1450
30	20	20	18	291	419	354	72–1584
0	20	20	7	564	612	—	191–1353

tributed. This was found to be the case, as is illustrated in Figure 1A, in which the frequency distribution of the initial eosinophile counts of 55 intact and 43 adrenalectomized rats on the normal diet is presented. Similar data is shown on a smaller number of intact and adrenalectomized rats on

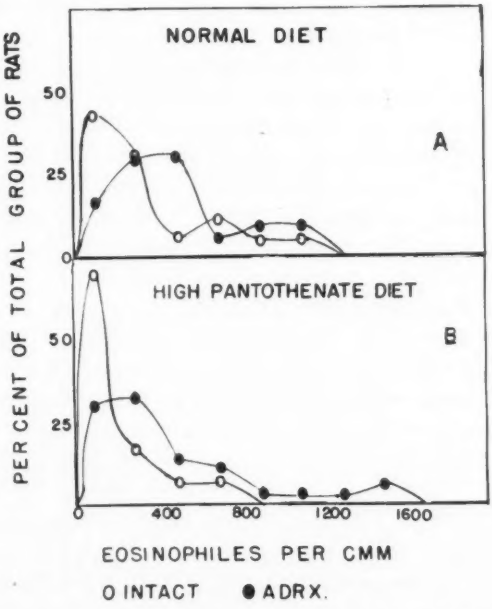


FIG. 1. The frequency distribution of resting eosinophile counts is shown for 55 intact and 43 adrenalectomized rats on the normal diet (1A) and for 30 intact and 35 adrenalectomized rats which received the high pantothenate diet for 7 to 20 days after a 30 day period of deficiency (1B). The per cent of the total number of counts in any group is shown on the ordinate and the eosinophiles per cmm. on the abscissa.

the high pantothenate diet (Fig. 1B). A marked skewing toward the lower values was observed in both groups of intact rats. After adrenalectomy, the distribution of the counts shifted toward the higher values. The change in the distribution of values after adrenalectomy in the rats on the normal diet was found to be highly significant by the chi-square test ($P<0.01$) (Johnson, 1949). A similar change in the distribution of eosinophile counts after adrenalectomy was observed in the other diet groups. However, the number of rats was smaller and statistical evaluation of the significance of the shift was attempted only in the rats on the normal diet.

Response of Eosinophiles to ACTH

Changes in the eosinophile count 4 hours after the injection of ACTH are summarized in Figure 2, in which data on 60 rats is presented. In order that the variation may be evaluated directly, the per cent change in the eosinophile count is shown for each rat. The dietary status of each group of animals is indicated in the figure. The decrease in the eosinophile counts of intact rats on a normal diet 4 hours after the injection of ACTH ranged from 5 to 80% of the initial value. In the adrenalectomized rats on the normal diet there was no significant change in the number of eosinophiles in 7 of the rats. In the remaining adrenalectomized rats the eosinophile count increased in one animal and decreased in 4 others. In rats on the pantothenate deficient diet it was sometimes difficult to obtain satisfactory blood samples, particularly after the injection of ACTH. In the 8 intact deficient rats in which studies were completed, the eosinophile response ranged from an increase of 80% to a decrease of 50% from the initial values. In 6 out of 7 adrenalectomized rats, increases in the eosinophile counts occurred after the injection of ACTH.

The intact and adrenalectomized animals receiving the high pantothenate supplement following the period of deficiency were studied after 7 or 20 days on the supplement and the same number of days after adrenalectomy. The response of the intact rats was approximately the same as that of intact animals on the normal diet, ranging from a 15 to an 85% decrease in the eosinophile count after 4 hours. In the adrenalectomized rats eosinophile counts in 3 animals were essentially unchanged 4 hours after the injection of ACTH, and in 2 rats they were significantly increased. After 20 days on the high pantothenate diet, there was a 20 to 65% decrease in the eosinophile counts in intact rats. In the adrenalectomized rats the values ranged from a decrease of 30% to an increase of 50% in one animal.

In Figure 3, the per cent change in the circulating eosinophiles 4 hours after the injection of cortisone is presented for 74 rats. In all diet groups the eosinophile counts decreased in both intact and adrenalectomized animals

to approximately the same degree. We have no explanation for the increased eosinophile level observed in 3 adrenalectomized rats on the normal diet, after the injection of cortisone (Fig. 3A). After 20 days on the high pantothenate diet (Fig. 3D), the eosinopenia following cortisone was somewhat less marked than in rats on a pantothenate deficient diet (Fig. 3B). In contrast to the effects of ACTH, the injection of cortisone caused an eosinopenia in all the pantothenate deficient rats (Fig. 3B).

All animals used in these experiments were accustomed to being handled. Even so, obtaining blood samples and injecting material intraperi-

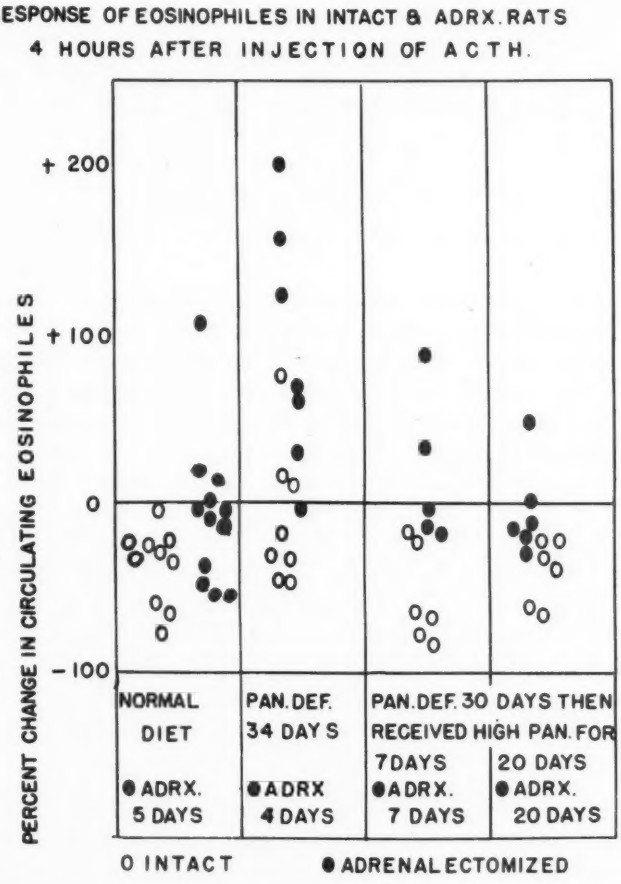


FIG. 2. The per cent change in the number of circulating eosinophiles 4 hours after the injection of 2 units of ACTH per 100 gm. body weight is shown for intact (o) and adrenalectomized (●) rats on the indicated diet. Each point represents one rat.

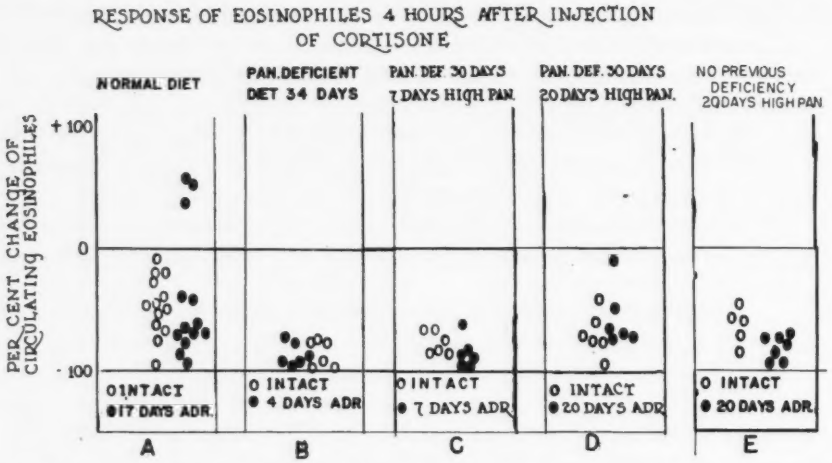

RESPONSE OF EOSINOPHILES 4 HOURS AFTER INJECTION OF CORTISONE
NORMAL DIET
PAN. DEFICIENT DIET 34 DAYS
PAN. DEF. 30 DAYS 7 DAYS HIGH PAN.
PAN. DEF. 30 DAYS 20 DAYS HIGH PAN.
NO PREVIOUS DEFICIENCY 20 DAYS HIGH PAN
PER CENT CHANGE OF CIRCULATING EOSINOPHILES
+100
0
-100
O INTACT ● 17 DAYS ADR.
O INTACT ● 4 DAYS ADR
O INTACT ● 7 DAYS ADR.
O INTACT ● 20 DAYS ADR.
O INTACT ● 20 DAYS ADR.
A
B
C
D
E

FIG. 3. The per cent change in the number of circulating eosinophiles 4 hours after the injection of 2 mg. of cortisone acetate per 100 gm. body weight is shown for intact (o) and adrenalectomized (●) rats on the indicated diet. Each point represents one rat.

toneally may be associated with both physical and emotional stress. Therefore, the effect of the procedures themselves on the eosinophile response was determined in both intact and adrenalectomized rats. The effect of the procedure on the eosinophile count, with and without an intraperitoneal injection of 0.5 c.c per 100 gm. of body weight, of 0.01 M acetate adjusted to pH 3.5 is given in Figure 4. Obtaining blood samples without any injection caused changes in the eosinophile count ranging from -55 to $+85\%$, in the intact rats. In the adrenalectomized rats the changes in the eosinophile counts varied from a decrease of -60% to an increase of $+320\%$. When 0.01 M acetate was injected (the solvent in which the ACTH was dissolved), changes in the eosinophile counts in intact rats ranged from $+48\%$ to -22%. In adrenalectomized rats the injection of acetate was associated with changes in the eosinophile counts ranging from 0 to $+55\%$. In confirmation of results reported by others (Sawyer and Parkerson, 1953), the injection of Nembutal was not found to be of value in stabilizing the eosinophile counts.

DISCUSSION

The wide variation observed in the number of eosinophiles in the rats prior to experimental procedures is similar to the results reported by other observers in this and other species. It has been reported that while adrenal insufficiency is often associated with high eosinophile counts, the variation is so great that differences may not be statistically significant (Recant *et*

al., 1950). The present data suggest that the eosinophile levels are significantly higher in adrenalectomized than in intact rats. However, this was only demonstrated by statistical methods which allowed for the skewed frequency distribution.

In each dietary group, there is an overlap between the results in intact and adrenalectomized rats following the injection of ACTH (Fig. 2). The

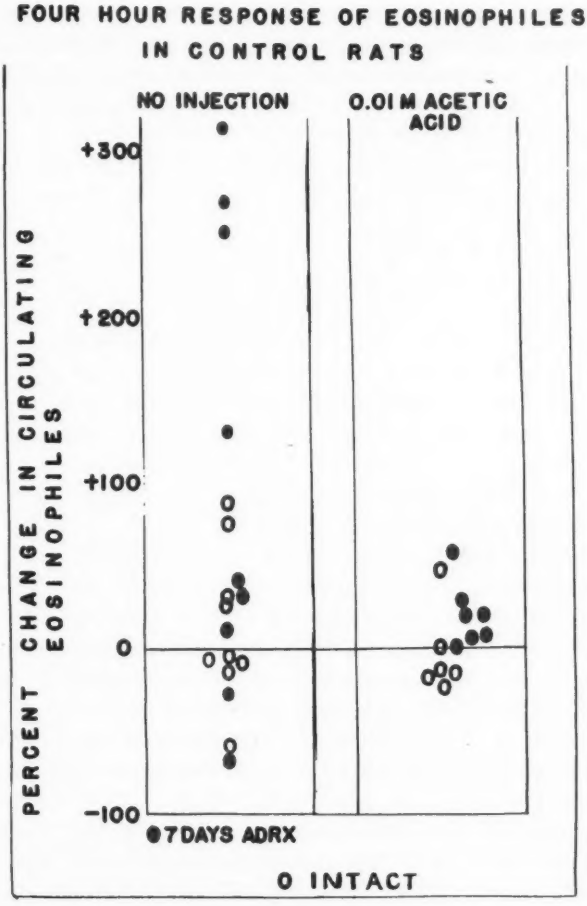


FIG. 4. The per cent change in the number of circulating eosinophiles is shown for intact (o) and adrenalectomized (●) rats 4 hours after taking a blood sample from the tail with no injection, and 4 hours after taking a blood sample and injecting a rat intraperitoneally with 0.5 cc. of 0.01 M acetate per 100 gm. body weight. Each point represents one rat.

variation in response may be partly due to the effect of the experimental procedures on the number of eosinophiles, as illustrated in Figure 4, and also to impurities of the ACTH preparation. An additional cause of variation in the number of eosinophiles is the counting procedures themselves, the errors of which have been reviewed by others (Berkson *et al.*, 1940; Best *et al.*, 1952).

The data presented (Fig. 2) tend to confirm the observations of Winters *et al.*, (1952) that pantothenate deficiency in the rat was associated with a diminished eosinophile response to ACTH. Neither swimming (Dumm *et al.*, 1949; Ralli, 1950; Dumm and Ralli, 1952), epinephrine (Winters *et al.*, 1952) nor ACTH (Dumm *et al.*, 1949; Ralli, 1950; Winters *et al.*, 1952) will regularly induce either a lymphopenia or an eosinopenia in rats on diets deficient in pantothenic acid. The effects of pantothenate deficiency on the eosinophile response of intact rats were largely overcome when the animals received liberal amounts of pantothenate for 7 days, and by 20 days the eosinophile response to ACTH was very similar to that of normal rats (Fig. 2). Pantothenic acid deficiency damages the cells of the adrenal cortex (Morgan and Simms 1939; Daft and Sebrell, 1939). The abnormal response of the white blood cells of intact pantothenate deficient rats to ACTH, epinephrine, or natural stress may be interpreted as reflecting the impairment in adrenal function resulting from the deficiency (Dumm *et al.*, 1949; Winters *et al.*, 1952). The fact that a significant eosinopenia followed the injection of cortisone in rats on all diets studied is consistent with this suggestion (Winters *et al.*, 1952). The possibility that changes in other tissues contribute to these results is not excluded.

In the adrenalectomized pantothenate deficient rats, the injection of ACTH was associated with marked increases in the number of eosinophiles (Fig. 2). This effect was less striking after the animals had received a high pantothenate diet for 7 days, and by 20 days only moderate changes in the eosinophile counts of adrenalectomized rats were observed comparable to those seen in adrenalectomized rats on a normal diet. The variations in the eosinophile response of individual animals was such that one could not judge the adrenal status of an animal on the basis of the effect of ACTH on the eosinophiles.

Other nutritional factors have been reported to influence the response of the eosinophiles to "stress." These include ascorbic acid and vitamin A (Bacchus and Altszuler, 1952; Tabor and Klopp, 1953). The possibility of a metabolic interrelation between pantothenic and ascorbic acids (Daft, 1951; Daft and Schwarz, 1952; Everson *et al.*, 1953; Hundley and Ing, 1953; Dumm and Ralli, 1949) suggests that changes in ascorbic acid metabolism may contribute to the changes in the eosinophile responses observed in pantothenate deficient animals.

In view of the variable responses of the eosinophiles to ACTH in the completely adrenalectomized rat, it is clear that changes in the number of circulating eosinophiles cannot be interpreted solely as an index of adrenal cortical function. The fact that cortisone was associated with a reasonably consistent eosinopenia should not be cited as evidence that the adrenal cortex is necessarily involved in the eosinophile response.

The effects induced by the parenteral administration of a hormone may differ from the effects produced by the natural secretion of the hormone. Since an eosinopenia can be produced in the completely adrenalectomized animal, it is clear that the production of an eosinopenia is not in itself evidence of adrenal cortical activity.

SUMMARY

Eosinophiles were counted by a direct chamber method in the tail blood of rats, on normal, pantothenate deficient and high pantothenate diets, before and 2, 4, and 6 hours after an injection of either ACTH or cortisone.

Resting eosinophile counts in rats on a normal diet varied widely and had a skewed frequency distribution. The increase in the number of eosinophiles in the adrenalectomized rats was statistically significant.

After 30 days on a pantothenate deficient diet the response of the eosinophile counts of intact rats to ACTH was diminished. In intact animals which had received a high pantothenate diet for 7 days following a period of deficiency, the eosinophile response was similar to that of normal rats.

The eosinophile counts of adrenalectomized pantothenate deficient rats tended to increase after the injection of ACTH. After 7 or 20 days on the high pantothenate diet, increases in the eosinophile counts after the injection of ACTH were less pronounced. Some overlap was encountered in the responses of intact and adrenalectomized rats to the injection of ACTH on all diets studied.

The injection of cortisone produced a significant eosinopenia in both intact and adrenalectomized rats on all diets studied.

The collection of blood samples was associated with significant changes in the eosinophile counts of both intact and adrenalectomized rats. These changes were less pronounced when the rats also received an intraperitoneal injection of 0.01 M acetate.

The data are interpreted as indicating that eosinophile counts can be modified by factors other than the hormones of the adrenal cortex.

Acknowledgment

We are indebted to Miss Elisabeth Street for her assistance in the statistical analysis of the data.

REFERENCES

BACCHUS, H. AND N. ALTSZULER: *Endocrinology* **51**: 1. 1952.
BERKSON, J., T. B. MAGATH AND M. HURN: *Am. J. Physiol.* **128**: 309. 1940.
BEST, W. R., R. C. MUEHRCKE AND R. M. KARK: *J. Clin. Investigation* **31**: 733. 1952.
DAFT, F. S.: *Fed. Proc.* **10**: 380. 1951.
DAFT, F. S. AND K. SCHWARZ: *Fed. Proc.* **11**: 200. 1952.
DAFT, F. S. AND W. H. SEBRELL: *U. S. Public Health Reports* **54**: 2247. 1939.
DUMM, M. E., P. OVANDO, P. ROTH AND E. P. RALLI: *Proc. Soc. Exper. Biol. & Med.* **71**: 368. 1949.
DUMM, M. E. AND E. P. RALLI: *Endocrinology* **45**: 188. 1949.
DUMM, M. E. AND E. P. RALLI: *Metabolism* **2**: 153. 1953.
EVERSON, G., L. NORTHROP, N. Y. CHUNG AND R. GETTY: *Fed. Proc.* **12**: 413. 1953.
HILLS, A. G., P. H. FORSHAM AND C. A. FINCH: *Blood* **3**: 755. 1948.
HUNDLEY, J. M. AND R. B. ING: *Fed. Proc.* **12**: 417. 1953.
JOHNSON, P. O.: *Statistical Methods in Research.* Prentice-Hall, Inc. New York. 1949.
MORGAN, A. F. AND H. D. SIMMS: *Science* **89**: 565. 1939.
RALLI, E. P.: *Adrenal Cortex, Trans. First Conf. New York:* Josiah Macy, Jr. Foundation, 173. 1950.
RECANT, L., D. M. HUME, P. H. FORSHAM AND G. W. THORN: *J. Clin. Endocrinology* **10**: 187. 1950.
SAWYER, C. H. AND G. R. PARKERSON, JR.: *Endocrinology* **52**: 346. 1953.
SPEIRS, R. S. AND R. K. MEYER: *Endocrinology* **45**: 403. 1949.
TABOR, C. W. AND C. T. KLOPP: *Fed. Proc.* **12**: 371. 1953.
WINTERS, R. W., R. B. SCHULTZ AND W. A. KREHL: *Endocrinology* **50**: 377. 1952.

ENHANCEMENT OF CORTICOTROPHIC ACTIVITY: EFFECT ON ADRENAL AND THYMUS WEIGHTS

HERMAN COHEN, HENRY H. FREEDMAN, WILLIAM KLEINBERG, NORMAN R. SCHECHTER, AND GUSTAV J. MARTIN

Princeton Laboratories, Inc., Princeton, New Jersey, and National Drug Company, Philadelphia, Pennsylvania

THE physiological utilization of subcutaneously or intramuscularly administered pituitary hormones has been shown to be greatly enhanced by means of depot agents, such as oil (Payne, *et al.*, 1951), gelatin (Wolfson and Robinson, 1952), polyvinylpyrrolidone (Pedersen-Bjergaard and Tonnesen, 1950), and others which delay absorption by a sequestering effect, or by chemical adducts such as tannates (Wilson and McGinty, 1951), protamine zinc (Solem, 1952), or alum precipitated proteins (Hajashida and Li, 1952) which tend to reduce the solubility of the proteins in the tissue fluids and thus delay their absorption into the blood stream. Recently (Cohen, *et al.*, 1953) data were presented by the authors which demonstrated that anti-hyaluronidase agents are effective in delaying the absorption and in enhancing the activity of adrenocorticotrophin. Coincidental anti-proteolytic action of these substances further enhanced such activity when the anti-hyaluronidase material together with the corticotrophin was injected in a protein base (gelatin). Adrenal ascorbic acid depletion was used as the index of effectiveness.

The data presented below are a continuation of our investigation, using the increase in adrenal weight and decrease in thymus weight as measures of adrenal cortical stimulation.

MATERIALS AND METHODS

Sprague-Dawley rats eight weeks old and weighing 110–130 grams were hypophysectomized by the parapharyngeal approach and rested for 5 days before the injections were started. Injections of adrenocorticotrophin dissolved in the appropriate vehicle were administered subcutaneously once daily in 0.5 ml. volume, for a period of 5 days. On the sixth day the animals were sacrificed and the adrenals and thymuses removed and weighed.

The following vehicles, all adjusted to pH 4.7, were studied:

1. 16% solution of Knox gelatin,
2. 16% solution of Knox gelatin in which was dissolved sufficient phosphorylated hesperidin to make a 4% solution of the latter,
3. Distilled water.

Received for publication July 14, 1953.

TABLE 1. THE EFFECT OF VEHICLE ON CORTICOTROPHIN-INDUCED CHANGES IN THE ADRENAL AND THYMUS GLANDS OF HYPOPHYSECTOMIZED RATS

Vehicle	No. of rats	Total dose* ACTH (USP units)	Adrenal weight, mg./100 g. body weight	Thymus weight, mg./100 g. body weight
16% gelatin +4% phosphorylated hesperidin	7	5	31.9 ± 1.0†	49 ± 6
16% gelatin	7	5	26.5 ± 1.3	113 ± 14
Distilled water	7	5	20.1 ± 1.0	171 ± 15
16% gelatin +4% phosphorylated hesperidin	5	0	15.6 ± 1.1	245 ± 19
Distilled water	5	0	13.1 ± 0.3	216 ± 22

* Purified adrenocorticotrophin having a potency of 25 USP units/mg. when assayed intravenously was incorporated into the solvents so that each ml. contained 2 USP units.
† Mean ± standard error.

RESULTS AND DISCUSSION

The data presented in Table 1 indicate the superiority of the gelatin vehicles in maintaining the secretory activity and the weight of the adrenal gland as compared to that of water. However, the addition of phosphorylated hesperidin, a potent anti-hyaluronidase and anti-proteolytic agent (Beiler and Martin, 1952), to the gelatin results in a marked enhancement of the activity of the adrenocorticotrophin dissolved therein. This enhancement is evidenced by a significant increase of adrenal weight over that obtained with the gelatin ACTH solution ($t > 3$), or with the aqueous ACTH solution ($t > 3$).

More striking evidence of the enhancing effect of the phosphorylated hesperidin is found in the degree of thymic involution. The ACTH in gelatin causes approximately a 50% decrease in thymic weight whereas ACTH in the gelatin-phosphorylated hesperidin vehicle reduces the weight of the thymus to approximately 20% of that of the control animals. The degree of thymic involution has been described as a more sensitive index of adrenocorticotrophic activity than the adrenal weight increase, and also is a reflection of the titer of circulating corticosteroids.

The mechanism by which phosphorylated hesperidin exerts its enhancing effect has been discussed in a prior publication (Cohen, *et al.*, 1953).

SUMMARY

Subcutaneous administration of purified adrenocorticotrophic hormone in gelatin and in gelatin plus phosphorylated hesperidin results in an enhanced effect of the hormone upon the adrenal cortex as evidenced by an increased adrenal weight and a decreased thymus weight, in hypophysectomized rats.

The combination of gelatin plus phosphorylated hesperidin is more

effective than gelatin alone in enhancing the effect of the corticotrophic hormone.

The action of the phosphorylated hesperidin is attributed to its anti-hyaluronidase and anti-proteolytic properties.

REFERENCES

BEILER, J. M. AND G. J. MARTIN: *J. Biol. Chem.* **174**: 31. 1952.

COHEN, H., H. H. FREEDMAN, W. KLEINBERG, M. EISLER AND G. J. MARTIN: *Proc. Soc. Exp. Biol. and Med.* **82**: 749. 1953.

HAJASHIDA, T. AND C. H. LI: *Endocrinology* **50**: 187. 1952.

PAYNE, R. W., I. N. ROSENBERG, M. S. RABEN, A. P. CLERONS AND E. B. ASTWOOD: *J. Clin. Invest.* **230**: 665. 1951.

PEDERSEN-BJERGAARD, K. AND M. TONNESEN: *Acta Endocrinol.* **5**: 270. 1950.

SOLEM, J. H.: *Lancet* **262**: 468. 1952.

WILSON, M. L. AND D. A. MCGINTY: *J. Clin. Endocrinol.* **11**: 963. 1951.

WOLFSON, W. Q. AND W. D. ROBINSON: *Fed. Proc.* **11**: 513. 1952.

THE PREVENTION OF ANEMIA IN HYPOPHYSECTOMIZED ADULT FEMALE RATS WITH COMBINED THYROXINE AND CORTISONE THERAPY[1]

ROGER C. CRAFTS[2]

Department of Anatomy, University of Cincinnati College of Medicine, Cincinnati, Ohio

ALTHOUGH it has been well established that hypophysectomy induces an anemia (see reviews by Gordon and Charipper, 1947, and Daughaday, Williams and Daland, 1948), the mechanism by which removal of the hypophysis causes this anemia is still a problem.

Many different means of therapy have been utilized in an attempt to prevent anemia in hypophysectomized rats, but the only ones that proved to be completely effective have been pituitary extracts (Flaks, Himmel, and Zoltnik, 1938; Contopoulos, Van Dyke, Simpson, Garcia, Huff, Williams and Evans, 1953), a combination of thyroxine and androgen (Crafts, unpublished data), a combination of thyroxine, androgen, and high protein diet (Crafts, 1949), cobalt nitrate (Crafts, 1952; Garcia, Van Dyke, and Berlin, 1952), and adrenocorticotrophic hormone (Garcia, Van Dyke, Huff, Elmlinger and Oda, 1951).

Thyroidectomy in rats has been shown to induce a moderate anemia but the anemia was not as severe as has been found after hypophysectomy (Crafts, 1941; Wilson, 1944; Gordon, Kadow, Finkelstein and Charipper, 1946). Adrenalectomy in rats has been shown to induce an anemia 10 to 20 days after removal of the glands. This anemia, however, disappeared. Sixty days after adrenalectomy, the blood picture was normal (Crafts, 1941; Piliero, Landau and Gordon, 1950; Gordon, Piliero and Landau, 1951). In spite of the fact that neither thyroidectomy nor adrenalectomy produced an anemia comparable to that which follows hypophysectomy, Crafts (1953a) has shown that a combined thyroidectomy and adrenalectomy in rats induces an anemia which mimics that found upon removal of the hypophysis.

The present experiment is an attempt to prevent anemia in hypophysectomized rats with thyroxine and cortisone therapy.

Received for publication July 14, 1953.

[1] Supported by a contract between the University of Cincinnati and the Office of Naval Research, Washington, D. C.

[2] With the technical assistance of Nancy H. Lancaster.

METHODS

Adult female rats of 3 to 4 months of age and of the Wistar strain were used in these experiments. All were fed standard "Purina chow" ad libitum and given lettuce once a week. Hypophysectomy was performed by the parapharyngeal approach, and at autopsy the completeness of the surgery was verified by careful examination of the sella turcica under a dissecting microscope. Blood was obtained by anesthetizing the rat with ether and cutting the tail. Five or 6 drops of blood were placed in a small vial containing a minute amount of heparin. Erythrocyte counts were done in duplicate using U. S. certified blood pipettes and the improved Neubauer counting chamber. Hemoglobin determinations were made in a Klett-Summerson photoelectric colorimeter. Hematocrit determinations were made with Van Allen hematocrit tubes (no diluent) and spun for one hour at 2000 r.p.m. Bone marrows were prepared in the following manner:—femurs were removed from the animal, both ends cut off, and one side of the shaft removed. This left the bone marrow in a "boat" of bone which was placed in Helly fixative. The bone marrow was removed from the bone after it had been left in 70% alcohol for 24 hours. Due to its firm consistency, it could be removed in a long strip. This was sectioned at 6 micra and stained with Giemsa stain.

RESULTS

This experiment includes data on 23 rats. Five served as normal controls, 9 were hypophysectomized, and 9 were hypophysectomized and received daily subcutaneous injections of thyroxine and cortisone. All animals were studied for 60 days after surgery, blood being examined at 10-day intervals. Data on erythrocyte counts are presented in Figure 1; hematocrit readings, Figure 2; and on hemoglobin levels, Figure 3. Values for mean corpuscular volume (M.C.V.),[3] mean corpuscular hemoglobin (M.C.H.),[4] and mean corpuscular hemoglobin concentration (M.C.H.C.)[5] are presented in Table 1. Observations on the bone marrow are included in the text.

Normal Controls

No significant changes occurred in the blood picture of the normal control rats. The blood picture in these animals before the experiment began was as follows:—erythrocyte count, 8.31 ± 0.95[6] million cells per cu. mm.; hematocrit reading, $43.4 \pm 1.2\%$; hemoglobin level, 16.4 ± 0.39 gm. per

---

[3] $\text{M.C.V.} = \dfrac{\text{vol. packed red cells, cc. per 1000 cc.}}{\text{erythrocyte count, millions per cu. mm.}}$.

[4] $\text{M.C.H.} = \dfrac{\text{hemoglobin, gm. per 1000 cc.}}{\text{erythrocyte count, millions per cu. mm.}}$.

[5] $\text{M.C.H.C.} = \dfrac{\text{hemoglobin, gm. per 100 cc.} \times 100}{\text{vol. packed red cells, cc. per 100 cc.}}$.

[6] Standard Deviation.

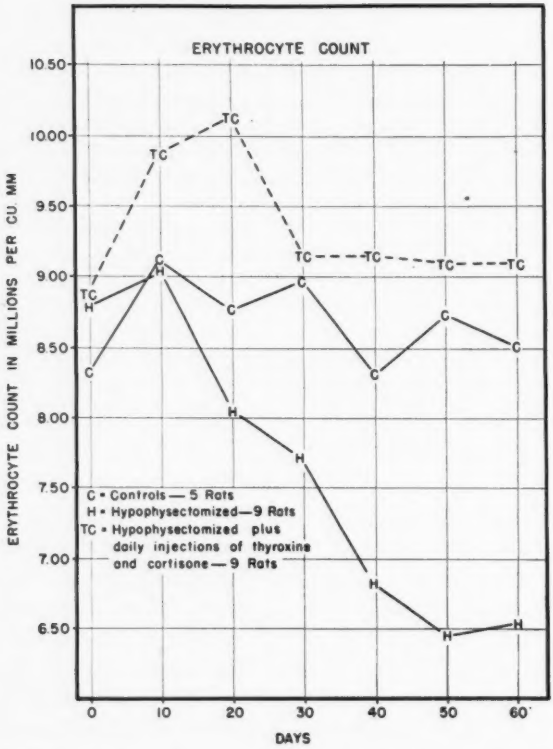


FIG. 1. The effects of hypophysectomy alone and hypophysectomy followed by daily injections of 0.005 mg. of thyroxine and 1.2 mg. of cortisone on erythrocyte counts in adult female rats. On day 21, dose of cortisone was reduced to 0.6 mg. per day.

100 cc of blood; mean corpuscular volume, 52.6 ± 5.3 cu. microns; mean corpuscular hemoglobin, 19.8 ± 1.9 micromicrograms; and mean corpuscular hemoglobin concentration, 37.7 ± 0.6 per cent. Sixty days later, at the termination of the experiment, these same normal control rats exhibited the following blood picture:—erythrocyte count, 8.51 ± 0.66 million cells per cu. mm.; hematocrit reading, $44.6 \pm 1.0\%$; hemoglobin level, 16.9 ± 0.53 gm. per 100 cc of blood; mean corpuscular volume, 52.6 ± 4.1 cu. microns; mean corpuscular hemoglobin, 19.9 ± 1.8 micromicrograms; and mean corpuscular hemoglobin concentration, 37.9 ± 0.8 per cent. The bone marrows were normal in appearance. The body weight in these control rats averaged 257 gm. before the experiment started. Sixty days later these rats averaged 273 gm. in body weight.

TABLE 1. THE EFFECTS OF HYPOPHYSECTOMY ALONE AND HYPOPHYSECTOMY FOLLOWED BY DAILY INJECTIONS OF 0.005 MG. OF THYROXINE AND 1.2 MG. OF CORTISONE ON THE MEAN CORPUSCULAR VOLUME (MCV), MEAN CORPUSCULAR HEMOGLOBIN (MCH), AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC) OF ADULT FEMALE RATS. ON DAY 21, DOSE OF CORTISONE WAS REDUCED TO 0.6 MG. PER DAY

Treatment	Day	M.C.V. in cubic microns	M.C.H. in micro-micrograms	M.C.H.C. in per cent
Normal Controls—No Treatment—5 rats	0	52.6 ±5.3	19.8 ±1.9	37.7 ±0.6
	60	52.6 ±4.1	19.9 ±1.8	37.9 ±0.8
Hypophysectomy—9 rats	0	50.4 ±3.7	18.6 ±1.2	36.9 ±1.6
	60	45.7 ±2.8	18.2 ±1.3	39.7 ±1.3
		$P = <0.01$	$P = >0.1$	$P = <0.001$
Hypophysectomy plus daily injections of thyroxine and cortisone—9 rats	0	52.1 ±4.7	18.4 ±1.4	35.3 ±1.0
	60	50.0 ±2.9	19.3 ±0.9	38.7 ±1.2
		$P = >0.1$	$P = >0.1$	$P = <0.001$

± = Standard Deviation.
P = Fisher's "t" test of significance.

Hypophysectomy

Hypophysectomy induced the usual anemia. The erythrocyte count in these hypophysectomized rats decreased from a pre-operative average of 8.82 ± 0.59 million cells per cu. mm. to an average of 6.55 ± 0.64 million cells 60 days later; the hematocrit reading, from an average of 44.5 ± 1.8 per cent to 29.8 ± 2.4 per cent; and the hemoglobin level, from an average of 16.4 ± 0.40 gm. per 100 cc of blood to an average of 11.8 ± 0.88 gm.

Hypophysectomy also induced a decrease in the average volume of each erythrocyte (M.C.V.—Table 1). This decrease from a pre-operative average of 50.4 ± 3.7 cu. microns to 45.7 ± 2.8 cu. microns on the 60th day of the experiment is a significant decrease ($P = <0.01$).[7] The anemia is, therefore, of the microcytic variety. In spite of this decrease in erythrocyte size, there was no decrease in the average hemoglobin content of each erythrocyte in these hypophysectomized animals (M.C.H.—Table 1). This is undoubtedly due to the significant ($P = <0.001$) increase in the concentration of hemoglobin in each erythrocyte (M.C.H.C.—Table 1) from a pre-operative average of $36.9 \pm 1.6\%$ to $39.7 \pm 1.3\%$.

[7] Fisher's "t" test of significance.

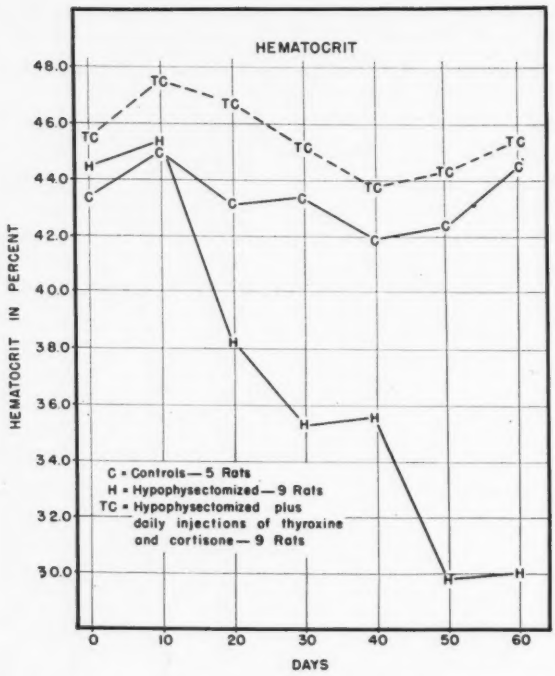


FIG. 2. The effects of hypophysectomy alone and hypophysectomy followed by daily injections of 0.005 mg. of thyroxine and 1.2 mg. of cortisone on hematocrit readings in adult female rats. On day 21, dose of cortisone was reduced to 0.6 mg. per day.

There was a variance in the bone marrow picture in these hypophysectomized animals. The usual finding of a decreased cellularity was not as marked in these rats as has been observed previously. The body weight of these hypophysectomized animals decreased from an average of 237 gm. before hypophysectomy to an average of 193 gm. 60 days later.

Hypophysectomy followed by thyroxine and cortisone therapy

Daily subcutaneous injections of 0.005 mg. of thyroxine[8] and 1.2 mg. of cortisone[9] (owing to the severe loss of body weight in these rats, on day 21 the dose of cortisone was decreased to 0.6 mg.) prevented anemia in hypophysectomized rats. Sixty days after hypophysectomy the erythrocyte count in these treated animals was 9.10 ± 0.68 million cells per cu. mm., the hematocrit reading, $45.3 \pm 1.9\%$ and the hemoglobin level,

[8] The thyroxine was generously furnished by the Organon Co., Inc.

[9] The cortisone was generously provided by Merck and Co., Inc.

17.6 ± 0.68 gm. per 100 cc. Although these figures are not significantly higher than the controls on the 60th day of the experiment, i.e. 8.51 million cells, 44.6%, and 16.9 gm. respectively, it should be noted (Fig. 1, 2, 3) that the averages for erythrocyte count, hematocrit reading, and hemoglobin level in these treated hypophysectomized rats were always higher than those for the control animals.

The slight decrease in average size of each erythrocyte in the hypophysectomized rats receiving thyroxine and cortisone (M.C.V.—Table 1) from a pre-operative average of 52.1 ± 4.7 cu. microns to 50.0 ± 2.9 cubic microns is not a significant change ($P = >0.1$), and the slight increase in average hemoglobin content of the erythrocytes in the same period of time (M.C.H.—Table 1) from 18.4 ± 1.4 micromicrograms to 19.3 ± 0.9 micromicrograms is also not significant ($P = >0.1$). Thyroxine and corti-

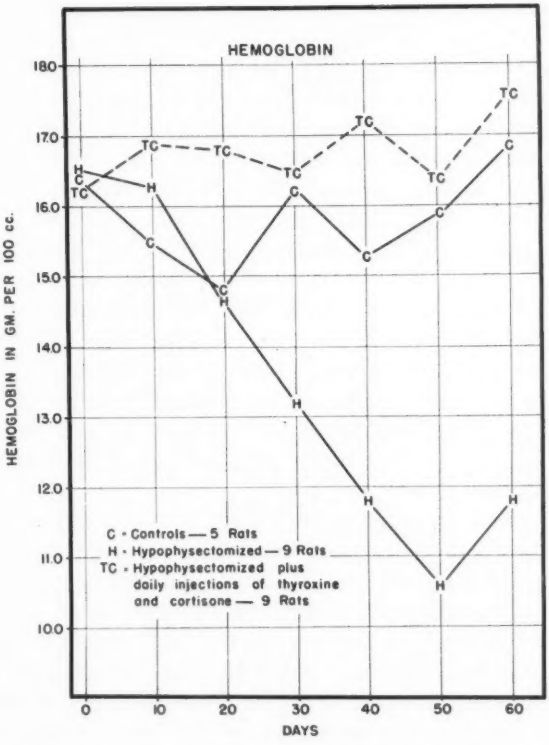


FIG. 3. The effects of hypophysectomy alone and hypophysectomy followed by daily injections of 0.005 mg. of thyroxine and 1.2 mg. of cortisone on hemoglobin levels in adult female rats. On day 21, dose of cortisone was reduced to 0.6 mg. per day.

sone therapy did induce an increase in hemoglobin concentration in the erythrocytes (M.C.H.C.—Table 1) from a pre-operative average of $35.3 \pm 1.0\%$ to 38.7 ± 1.2 per cent 60 days later. This is a significant change ($P = <0.001$).

The bone marrows of the hypophysectomized rats treated with thyroxine and cortisone exhibited an increase in fat content and a decrease in cellularity. In spite of these changes, erythropoiesis was active and there seemed to be an increase in younger forms of the erythroid series. Differential counts, however, have not been made.

The weight of these animals decreased from an average of 229 gm. before hypophysectomy to 149 gm. on the 20th day of treatment. Halving the daily dose of cortisone on the 21st day of treatment induced a gradual increase in average body weight to 157 gm. on the 60th day of the experiment.

DISCUSSION

A combined treatment of thyroxine and cortisone prevented the anemia which ordinarily follows removal of the hypophysis. This finding, coupled with the previous report that a combined thyroidectomy and adrenalectomy induced an anemia which duplicated that induced by hypophysectomy (Crafts, 1953a), would indicate that there is certainly a possibility that anemia in hypophysectomized rats is due to decreased activity of the thyroid and adrenal glands following removal of the hypophysis.

These results would seem to substantiate the hypothesis that anemia in hypophysectomized animals is due to faulty metabolism. The role of the thyroid gland in metabolism is unquestioned, and the adrenal cortex certainly plays a role in carbohydrate metabolism. In addition, it has been shown that anemia in hypophysectomized rats can be prevented by a combined therapy of thyroxine, testosterone propionate, and a high protein diet (Crafts, 1949).

The fact that anemia in hypophysectomized rats could not be prevented with growth hormone in dose levels which stimulated growth (Meyer, Stewart, Thewlis and Rusch, 1937; Gaebler and Mathies, 1951; Van Dyke, Simpson, Garcia and Evans, 1952; Crafts, 1953b), casts suspicion on the role of the high protein diet in the prevention of anemia in hypophysectomized rats as mentioned above. Accordingly, hypophysectomized rats were treated with thyroxine and testosterone propionate and the effects on the blood picture compared to treatment with a high protein diet in addition to thyroxine and testosterone propionate (Crafts, unpublished data). The anemia was prevented in hypophysectomized rats with thyroxine and testosterone but addition of a high protein diet actually caused

an increase in the erythrocyte count to levels above normal. Further work is necessary to determine the role played by faulty metabolism in the development of the anemia which is induced by hypophysectomy.

The appearance of the bone marrow of the rats in the present experiment deserves comment. In this investigator's experience, whenever therapy prevented anemia in hypophysectomized rats, there was a decrease in fat content and an increase in cellularity of the marrow. Conversely, hypophysectomy was followed by an increase in marrow fat and a decreased cellularity. In the present experiment, however, the hypophysectomized animals which received no treatment varied considerably as to fat content and cellularity of the bone marrow. In addition, the bone marrows of the hypophysectomized rats treated with thyroxine and cortisone exhibited a marked increase in fat content. These results are similar to those reported by Gordon, Piliero and Landau (1951) in which they found that cortisone increased the "vacuolization" and decreased the total cellularity of the marrow of adrenalectomized rats. Similar results were obtained by Baker and Ingle (1948) with ACTH therapy. As Gordon and co-workers pointed out, this increase in marrow fat may be due to the generalized increase in fat and decrease in weight of animals treated with cortisone. In spite of this increase in marrow fat and decreased marrow cellularity after cortisone injections, the cells present in the bone marrow were capable of producing an adequate number of erythrocytes in the adrenalectomized rat (Gordon and co-workers, 1951) or in hypophysectomized animals as shown in the present experiment.

Anemia in hypophysectomized rats has been duplicated by a combined removal of the thyroid and adrenal glands, and has been prevented by daily subcutaneous injections of thyroxine and cortisone. These results support the contention that anemia in hypophysectomized rats is due to faulty metabolism resulting from a combined hypothyroidism and hypoadrenocorticalism.

SUMMARY

One group of adult female rats of the Wistar strain was hypophysectomized and given daily subcutaneous injections of 0.005 mg. of thyroxine and 1.2 mg. of cortisone (on day 21, cortisone was decreased to 0.6 mg. per day), while another group was hypophysectomized and given no therapy. Blood was studied at 10-day intervals for 60 days.

Hypophysectomy induced a decrease in erythrocyte count from 8.82 $\pm$0.59 million cells per cu. mm. before hypophysectomy to 6.55$\pm$0.64 million cells 60 days later; in hematocrit reading, from 44.5$\pm$1.8% to 29.8$\pm$2.4%; and in hemoglobin level, from 16.4$\pm$0.40 gm. per 100 cc of blood to 11.8$\pm$0.88 gm. In addition, the mean corpuscular volume in these

untreated hypophysectomized rats decreased and the mean corpuscular hemoglobin concentration increased.

Thyroxine and cortisone injections in hypophysectomized rats prevented this anemia. On the 60th day of the experiment, the erythrocyte count in the treated hypophysectomized rats was 9.40 ± 0.68 million cells per cu. mm.; the hematocrit reading, $45.3 \pm 1.9\%$; and the hemoglobin level, 17.6 ± 0.68 gm. per 100 cc of blood. There was no significant decrease in mean corpuscular volume in these animals but the mean corpuscular hemoglobin concentration increased. Although the bone marrows of these hypophysectomized treated rats exhibited an increase in fat and a decreased total cellularity, erythropoiesis was active.

REFERENCES

BAKER, B. L. AND D. J. INGLE: *Endocrinology* **43**: 422. 1948.

CONTOPOULOS, A. N., D. C. VAN DYKE, M. E. SIMPSON, J. F. GARCIA, R. L. HUFF, B. S. WILLIAMS AND H. M. EVANS: *Blood* **8**: 131. 1953.

CRAFTS, R. C.: *Endocrinology* **29**: 596. 1941.

CRAFTS, R. C.: *Endocrinology* **45**: 159. 1949.

CRAFTS, R. C.: *Blood* **7**: 863. 1952.

CRAFTS, R. C.: *Endocrinology* **53**: 465. 1953a.

CRAFTS, R. C.: *Endocrinology* **53**: 235. 1953b.

FLAKS, J., I. HIMMEL AND A. ZLOTNIK: *Presse Med.* **46**: 1506. 1938.

GAEBLER, O. H. AND J. C. MATHIES: *Endocrinology* **48**: 623. 1951.

GARCIA, J. F., D. C. VAN DYKE, R. L. HUFF, P. J. ELMLINGER AND J. M. ODA: *Proc. Soc. Exper. Biol. & Med.* **76**: 707. 1951.

GARCIA, J. F., D. C. VAN DYKE AND N. I. BERLIN: *Proc. Soc. Exper. Biol. & Med.* **80**: 472. 1952.

GORDON, A. S., P. C. KADOW, G. FINKELSTEIN AND H. A. CHARIPPER: *Am. J. Med. Sci.* **212**: 385. 1946.

GORDON, A. S., S. L. PILIERO AND D. LANDAU: *Endocrinology* **49**: 497. 1951.

MEYER, O. O., G. E. STEWART, E. W. THEWLIS AND H. P. RUSCH: *Folia Hematologica* **57**: 99. 1937.

PILIERO, S. L., D. LANDAU AND A. S. GORDON: *Science* **112**: 559. 1950.

VAN DYKE, D. C., M. E. SIMPSON, J. F. GARCIA AND H. M. EVANS: *Proc. Soc. Exper. Biol. & Med.* **81**: 574. 1952.

WILSON, T. E.: *Med. J. Australia* **31**: 261. 1944.

THE EFFECT OF GROWTH-INHIBITING CONDITIONS UPON THE LEVATOR ANI MUSCLE OF THE RAT

EUGENE EISENBERG AND GILBERT S. GORDAN

The Endocrine Clinic of the Department of Medicine, University of California School of Medicine, San Francisco, California

IN PREVIOUS studies, growth of the levator ani muscle of the castrate immature male rat was induced by various anabolic agents. It was found that the amount of growth produced paralleled the known anabolic potency of these agents (Eisenberg and Gordan 1950). For this reason, the weight of the levator ani muscle of the castrate male rat was used as a gauge of the protein-anabolic activity of steroids. Purified pituitary growth extract also causes the muscle to grow. This observation suggested that the muscle is an index of changes in nitrogen balance induced by steroids and other growth-promoting substances.

The present study was undertaken to determine whether the levator ani muscle also reflects the loss of protein that accompanies growth-inhibiting conditions. Because Rupp, Paschkis, and Cantarow (1949) have shown that small doses of thyroid hormone are anabolic in hypothyroid rats, while large doses are catabolic, the weight of the levator ani muscle was measured in hypothyroidism, in hypothyroidism remedied by the administration of small doses of thyroid substance, and in severe hyperthyroidism. The effect of administration of the catabolic steroid, cortisone, was also studied.

METHOD

The animals, all male rats of the Long-Evans strain, were divided into seven groups: 1) normal controls, 2) hypothyroid rats with intact gonads, 3) hyperthyroid rats with intact gonads, 4) castrate controls, 5) castrate hypothyroid animals, 6) castrate hypothyroid rats treated with 2 mg. desiccated thyroid substance daily from the fifty-third to the sixtieth day of life, and 7) castrates treated with cortisone. The castrations were performed on the thirtieth day of the animal's life, when the body weight of the animals in each group average 73 $\pm$4.0 gm.

Hypothyroidism was induced by the addition of 0.1% propylthiouracil to the drinking water during the second month of the animal's life. Hyperthyroidism was produced by feeding 30 mg. of thyroid substance every other day from the 35th to the 60th day of age. This dose resulted in basal metabolic rates of plus 65 to plus 100%. The oxygen consumption of slices of liver from these animals (measured *in vitro* by the direct method of Warburg) was exactly double that of liver slices from normal animals. The rats

Received for publication July 15, 1953.

treated with cortisone received 0.1 mg. of the steroid in sesame oil subcutaneously for seven days preceding autopsy.

All animals were killed by decapitation on the sixtieth day of life. Dissection of the levator ani muscle and the seminal vesicles was carried out as described earlier (Eisenberg and Gordan 1950).

RESULTS

The data are summarized in Table 1. It will be noted that the body weight attained by hypothyroid and hyperthyroid rats with intact gonads was less than that of the normal controls. In these hypothyroid and hyperthyroid animals, the weight of the levator ani muscle and of the seminal vesicles was also less than normal. In the hyperthyroid group, body weight and weight of the levator ani were both lower than those of castrate rats, in fact, approximately normal for animals 30 days of age (Eisenberg and Gordan 1950). The seminal vesicles, however, weighed more than $2\frac{1}{2}$ times as much in these animals as in castrates.

In the castrate-hypothyroid group, the average body weight was lower than that of either the intact or the castrate controls, but not significantly less than the weight of hypothyroid animals with intact gonads. The levator ani muscles in the castrate-hypothyroid group weighed only slightly less than those of castrate control rats, but only one-half as much as those of hypothyroid rats which were not castrated. Administration of small amounts of thyroid substance to the castrate hypothyroid rats resulted in restoration of the weight of the levator ani muscle and in gain of body weight, but had no effect on the weight of the seminal vesicles. Cortisone caused further atrophy of the already atrophic levator ani muscle of the castrate rats ($p < 0.01$), but effected no change in the weight of the seminal vesicles.

DISCUSSION

Considerable divergence from a euthyroid state in the directions of hypothyroidism and hyperthyroidism has been shown to be associated

TABLE 1. EFFECT OF GROWTH-INHIBITING CONDITIONS UPON BODY WEIGHT, WEIGHT OF LEVATOR ANI MUSCLES AND OF SEMINAL VESICLES OF MALE RATS

No. of rats	Group	Body weight (gm. $\pm\sigma_m$.)	Levator ani (mgm. $\pm\sigma_m$.)	Seminal vesicle (mgm. $\pm\sigma_m$.)
21	Normal control	218 ±7.9	101 ±6.3	276 ±15.5
10	Hypothyroid (not castrated)	139 ±4.3	58.0 ±8.0	117 ±30.0
13	Hyperthyroid (not castrated)	99 ±3.2	22.8 ±5.1	37.8 ± 6.3
16	Castrate control	179 ±4.8	33 ±2.9	14 ± 1.2
10	Castrate hypothyroid	127 ±3.2	29 ±1.0	13 ± 1.0
10	Thyroid treated hypothyroid castrates	155 ±4.1	36 ±1.4	14 ± 1.0
9	Cortisone-treated castrates	168 ±6.2	23 ±3.0	13 ± 1.1

with a negative nitrogen balance (Rupp, Paschkis, and Cantarow, 1949). Likewise, the protein-catabolic[1] effects of cortisone are well-known (Wells and Kendall, 1940; Ingle, Prestrud and Rice, 1950). The data presented above show that the weight of the levator ani muscle reflects these changes.

A previous study showed that testosterone compounds stimulate the muscle to hypertrophy in the intact animal (Eisenberg and Gordan, 1950). This observation was recently confirmed by Scow (1952). For assay of anabolic activity, we thought the castrate male rat, with its atrophic musculature, would be a more sensitive preparation than the intact animal. In the present experiment, the weight of the levator ani muscle of the castrate male rat also reflected changes in the direction of catabolism, as evidenced by the animals that received cortisone. It will be seen, however, that the weight of the muscle of the non-castrate hypothyroid rats is 43% less than that of the normal control rats, while the muscle weight of the castrate hypothyroid animals is only 13% less than that of the castrate controls. The greater sensitivity of the intact animals to these catabolic influences suggests that they are more suitable for the assay of protein-catabolic activity.

We believe that the growth of the levator ani muscle is not solely an androgenic phenomenon, since: 1) The muscle is stimulated to grow in the hypothyroid castrate animal by treatment with thyroid substance alone; 2) The small muscle of the castrate animal is rendered even more atrophic by administration of cortisone; 3) We have previously shown that the muscle grows in the growing animal deprived of its gonads (Sakamoto, Gordan, and Eisenberg, 1951); 4) We have also shown that pituitary growth hormone stimulates growth of this muscle in the castrate rat; 5) The muscle is also stimulated to grow by non-androgenic doses of methyl-androstenediol (Gordan, Eisenberg, Moon, and Sakamoto, 1951), or by administration of androstadiene Δ1–2, 4–5 dione 3,17 and androstadiene Δ4–5, 6–7 dione 3,17 and testololactone (Shemano, Gordan, and Eisenberg, 1951). In all these instances, lack of androgenic activity was demonstrated by the constant weight of the seminal vesicles.[2]

SUMMARY

The effect of known protein-catabolic conditions on the levator ani muscle of male rats has been studied. The growth-inhibiting states studied

[1] Whether the effects described are catabolic or anti-anabolic cannot be determined from these data. The net result of either is decreased bulk of protein tissue.

[2] Subsequent to the preparation of this paper, Hershberger, Shipley, and Meyer have reported that 19-nortestosterone and other 19-nor analogs of androgens are strongly myotrophic and only weakly androgenic (Proc. Soc. Exper. Biol. and Med. **83**: 175, 1953).

were hypothyroidism, severe hyperthyroidism, castration, and administration of cortisone. In these conditions it was found that the levator ani muscle of the male rat reflects the general loss of body tissue. Administration of thyroid substance restores to the castrate level the atrophic levator ani muscle of the castrate hypothyroid rat.

The levator ani muscle of the *normal* male rat is a more responsive assay modality for protein catabolism than the muscle of the *castrate* rat.

Responsiveness of the levator ani muscle to agents free of androgenic activity is again demonstrated.

REFERENCES

Eisenberg, E. and G. S. Gordan: *J. Pharmacol. & Exper. Therap.* **99**: 38. 1950.

Gordan, G. S., E. Eisenberg, H. D. Moon and W. Sakamoto: *J. Clin. Endocrinol.* **11**: 209. 1951.

Ingle, D. J., M. C. Prestrud and K. L. Rice: *Endocrinology* **46**: 510. 1950.

Rupp, J., K. E. Paschkis and A. Cantarow: *Endocrinology* **44**: 449. 1949.

Sakamoto, W., G. S. Gordan and E. Eisenberg: *Proc. Soc. Exper. Biol. and Med.* **76**: 406. 1951.

Scow, R. O.: *Endocrinology* **51**: 42. 1952.

Shemano, I., G. S. Gordan and E. Eisenberg: *Proc. Soc. Exper. Biol. and Med.* **78**: 612. 1951.

Wells, B. B. and E. C. Kendall: *Proc., Staff Meets. Mayo Clinic* **15**: 324. 1940.

THYROIDAL IODIDE TRAPPING AS INFLUENCED BY SERUM IODIDE LEVELS AND THYROTROPHIN[1,2]

N. S. HALMI

Department of Anatomy, State University of Iowa, Iowa City, Iowa

IT IS well established that the thyroid possesses a mechanism whereby it concentrates ("traps") inorganic iodide (VanderLaan and VanderLaan, 1947; Taurog *et al.*, 1947). Studies using tracer doses of radioactive iodine have disclosed the thyroid:serum iodide gradient to be profoundly influenced by thyrotrophin (TSH) (VanderLaan and VanderLaan, 1947; VanderLaan and Greer, 1950; Ingbar and Roberts, 1952; Halmi *et al.*, 1953). Up to 100 μg of stable iodide carrier added to the tracer had no appreciable effect on the thyroid:serum radioiodide concentration ratio (T/S) of normal (VanderLaan and VanderLaan, 1947) or of goitrous rats (VanderLaan and VanderLaan, 1947; Taurog *et al.*, 1947). However, further increase of the dose of stable iodide caused the T/S of propylthiouracil (PTU)-treated rats to drop (VanderLaan and VanderLaan, 1947; Wyngaarden *et al.*, 1953). This suggested to Raben and Astwood (1949) that the capacity of the thyroid for iodide is limited, and indeed, the early work of Leblond and Süe (1941) with I^{128} given in sufficiently high doses to interfere with organic binding had shown that the iodide collecting mechanism of the guinea pig thyroid becomes "saturated" when the gland contains 16 mg% of iodine.

The present study was undertaken to compare the effect of graded doses of carrier iodide on the T/S of hypophysectomized, normal, hypophysectomized-TSH treated and PTU-fed rats and to estimate the capacity of the thyroidal iodide "trap" in these groups of animals.

MATERIALS AND METHODS

Several groups of young adult male Sprague-Dawley rats were used in this study. The untreated animals weighed 200–250 gm. and were fed Rockland pellets. Rats of similar size received 0.03% propylthiouracil (PTU) in ground Rockland diet for 20 days. Hypophysectomized rats (100–150 gm.) obtained from the Hormone Assay Laboratories, Chicago, were used 10 days after operation. When TSH was administered to them, it was

Received for publication July 20, 1953.

[1] Supported by a grant from the Central Scientific Fund of the College of Medicine, State University of Iowa.

[2] The assistance of Mr. Meredith R. Saunders during part of this study is gratefully acknowledged.

given in the form of a single subcutaneous injection (0.25 USP units = 5 mg. USP Thyrotropin Reference Substance) 24 hrs. before the administration of I^{131}.

All rats received a subcutaneous injection of 6 mg. PTU in alkaline solution in order to block thyroid hormone synthesis. Some animals were simultaneously given 100 mg. KSCN by the same route. Forty-five minutes later the rats were injected subcutaneously with 1 ml. of water containing 2–10 μc. of radioiodide without or with added stable sodium iodide (100 μg. to 200 mg.). After an interval of 1 to 1½ hrs. the animals were anesthetized with ether and exsanguinated via the abdominal aorta. Thyroid-serum iodide concentration ratio determinations were carried out as described in our previous study (Halmi *et al.*, 1953). The specific activity of the injected solutions was also determined by means of the thin mica-window Geiger-Müller counter and the concentration of injected I^{127} in thyroids and sera calculated on the basis of this measurement and that of their radioactivity. Since large amounts of I^{127} were given, the original stable blood and thyroid iodide was not taken into consideration. When thyroids were studied histologically, they were fixed in SUSA, embedded in paraffin, cut at 6 μ and stained with Gomori's trichrome.

RESULTS

The results of the experiments are summarized in Table 1. Figure 1 is a logarithmic plot of the thyroid:serum iodide concentration ratios (T/S) against the serum iodide levels. It can be seen that 100 μg. of carrier NaI had no significant effect on the T/S in any of the groups, although the

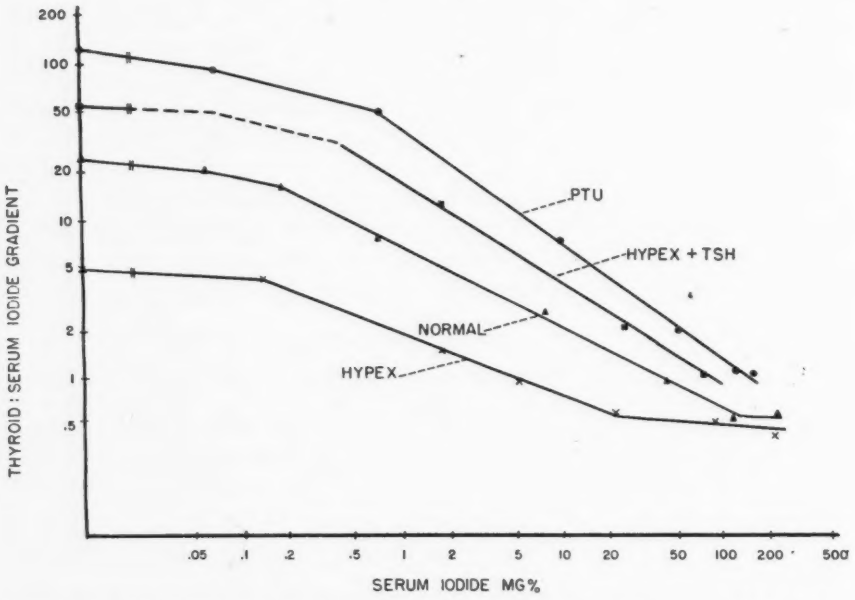


FIG. 1. Logarithmic plot of thyroid: serum iodide gradients against serum iodide levels.

TABLE 1. THYROID:SERUM IODIDE CONCENTRATION (T/S) RATIOS, THYROID AND SERUM IODIDE CONCENTRATIONS AND ESTIMATED THYROIDAL "TRAPPED" IODIDE CONCENTRATIONS IN HYPOPHYSECTOMIZED, NORMAL, HYPOPHYSECTOMIZED-TSH TREATED AND PROPYLTHIOURACIL (PTU) FED RATS 1—1½ HRS. AFTER THE INJECTION OF GRADED DOSES OF I^{131}-LABELLED STABLE IODIDE

Group	Dose of Na I^{127}, mg.	No. rats	T/S ratio	T Mg. of I^{127} per 100 gm. thyroid	S Mg. of I^{127} per 100 ml. serum		T minus S/2.5 Mg. of "trapped" I^{127} per 100 gm. thyroid	
Hypophysectomized	0	12	5.1 ± .51*	—	—		—	
	.1	12	4.2 ± .50	.52 ± .08	.13 ± .01		.48 ± .07	
	1.0	8	1.5 ± .22	2.3 ± .22	1.6 ± .22		1.7 ± .23	
	3.0	4	.95 ± .06	4.8 ± .58	5.0 ± .76		2.8 ± .41	
	10.0	7	.57 ± .04	11.5 ± .93	21.8 ± 2.3		3.3 ± .94	
	50.0	6	.49 ± .05	41.9 ± 2.7	88.8 ± 4.4	143.6 ± 17.7†	6.8 ± 5.6	2.3 ± 11.1†
	100.0	5	.39 ± .03	80.2 ± 5.1	207.5 ± 13.7		−3.1 ± 6.2	
Normal	0	8	24.7 ± 1.6	—	—		—	
	.1	4	20.6 ± 1.8	1.3 ± .05	.06 ± .01		1.3 ± .05	
	.3	8	17.0 ± 2.1	3.0 ± .30	.18 ± .01		2.9 ± .30	
	1.0	7	8.0 ± .91	5.8 ± .75	.72 ± .03		5.4 ± .92	
	10.0	4	2.6 ± .10	20.1 ± 1.1	7.8 ± .40		17.0 ± 1.0	
	50.0	8	.94 ± .04	39.3 ± 4.6	44.2 ± 4.2		21.6 ± 4.4	
	100.0	7	.51 ± .02	60.1 ± 2.3	118.4 ± 4.6	154.4 ± 15.0†	12.6 ± 3.1	19.0 ± 14.9†
	200.0	4	.55 ± .08	117.2 ± 13.2	218.0 ± 16.4		30.1 ± 13.6	
Hypophysectomized TSH treated	0	6	55.5 ± 9.5	—	—		—	
	1.0	6	13.7 ± 2.3	23.1 ± 2.6	1.8 ± .18		22.3 ± 2.5	
	10.0	5	2.1 ± .30	47.4 ± 4.4	23.6 ± 2.8		37.9 ± 4.2	
	50.0	4	1.0 ± .08	72.3 ± 5.8	72.5 ± 3.3		43.3 ± 5.3	
PTU fed	0	6	127.3 ± 11.1	—	—		—	
	.1	7	92.9 ± 8.6	6.8 ± .54	.07 ± .01		6.8 ± .54	
	1.0	6	47.5 ± 3.4	35.5 ± 1.5	.75 ± .06		35.2 ± 2.3	
	10.0	7	7.3 ± .38	74.8 ± 2.3	10.3 ± .35		70.6 ± 2.2	
	40.0	6	1.9 ± .24	96.0 ± 10.2	51.6 ± 3.0		75.3 ± 10.6	
	100.0	6	1.05 ± .07	124.8 ± 6.6	121.4 ± 5.2		76.0 ± 7.3	
	200.0	4	.96 ± .06	149.4 ± 5.5	157.7 ± 9.0		86.3 ± 5.5	

* Mean ± standard error.

† Mean ± standard error for pooled groups. The curves in Fig. 2 are drawn on the basis of these values.

values tended to be lower than in the animals given tracer doses of I^{131} only. Further increase of the serum iodide levels caused the T/S to drop gradually to 1 or less in every group. The serum idoide concentrations which were necessary to eliminate the thyroid:serum iodide gradient depended on the level of thyrotrophic stimulation and were of the following order of magnitude: 4–5 mg% for hypophysectomized rats, 35–40 mg% for normal rats, 70–75 mg% for hypophysectomized rats given TSH and 130–140 mg% for PTU treated animals.

Depression of the T/S below 1 was not attempted in the goitrous and the TSH treated hypophysectomized rats. In the normal and the untreated hypophysectomized group, however, a drop of the T/S to values lower than unity was observed when the serum iodide concentration was sufficiently raised. In the hypophysectomized animals the curve of the T/S appeared to level off at 0.4, in the normal rats at 0.5 (Fig. 1). In five normal rats which had received 100 mg. of KSCN the T/S was 0.66 ± 0.03. Another group of five normal rats given 100 mg. of KSCN and 100 mg. of NaI^{127} had a T/S of 0.55 ± 0.08.

The concentration of I^{127} in the thyroids rose progressively in all groups as the serum iodide levels were elevated. This is not surprising since the thyroid contains blood and a diffusion space for iodide in addition to the parenchymal mechanism which "traps" iodide and which presumably can become "saturated." As the "trapped" iodide in the thyroids of hypophysectomized rats given high doses of stable iodide can be considered to constitute a negligible portion of the total iodide, the minimum T/S value of about 0.4 in this condition appears to indicate that approximately 40% of the thyroid volume constitutes the intra- and extravascular iodide diffusion space of this organ. In order to estimate the concentration of "trapped" iodide within the thyroid, 40% of the serum iodide concentration value thus has to be subtracted from the total thyroidal iodide concentration. "Trapped" iodide concentrations calculated in this manner appeared to reach a plateau with increasing serum iodide levels in all groups of rats studied (Table 1, Fig. 2). It is also evident that TSH had a similar effect on the capacity of the thyroidal iodide "trap" as it had on the T/S values in experiments using tracer doses of carrier-free radioiodide. Notably, the "saturation" levels of the "trapping" mechanism appeared to be of the following order of magnitude in our experimental groups: 3 mg. of iodide per 100 gm. thyroid tissue in hypophysectomized rats, 20 mg. in normal animals, 40–45 mg. in hypophysectomized-TSH treated rats and 80–85 mg. in PTU treated animals. Figure 3 shows that TSH administration failed to alter the morphological picture of the thyroid in hypophysectomized rats, and yet it imparted to these glands a greater capacity for "trapped" iodide than that of the histologically more active appearing thyroids of normal animals.

It is evident from Table 1 that a significant decline of the T/S occurred with rising serum iodide concentrations in all groups *before* "saturation" of the iodide "trapping" mechanism was achieved.

DISCUSSION

In an earlier study we had reported that the T/S ratio is lower in intact rats given large doses of thyroxin (1.6) and in hypophysectomized-thyroxin treated animals (3.9) than in untreated hypophysectomized rats (6.3-6.9) (Halmi *et al.*,1953). Since these results were obtained with pharmacological amounts of thyroxin, there could be some doubt whether they actually signify that thyroxin exerts a direct depressing effect on the thyroidal iodide concentrating mechanism or whether they were due to dilution of the injected tracer by stable iodide derived from the injected thyroxin. The

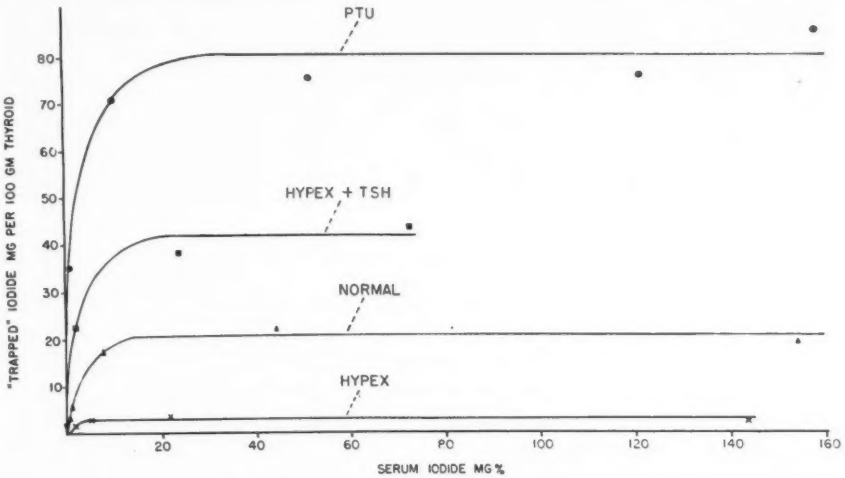


FIG. 2. Estimated thyroidal "trapped" iodide concentrations (total thyroidal iodide concentration minus 40% of serum iodide concentration) plotted against serum iodide levels.

present study indicates that the latter explanation is unlikely. Deiodination of thyroxin *in vivo* is a sluggish process (Gross and Leblond, 1947; Johnson and Albert, 1951) and, in the experiments referred to, could not have resulted in sufficiently high levels of serum iodide at the time of the T/S determination to account for the observed depression of the thyroid:serum iodide gradient. However, it cannot be stated with certainty that thyroxin *as such* acts on the thyroidal iodide "trap," and the effect of chronic elevation of serum iodide levels on this mechanism remains to be investigated.

Iodide has been used for the estimation of the extracellular space (Wallace and Brodie, 1937), although Leblond (1942) suggests that it penetrates

to some extent into all cells. Our estimate of the thyroidal iodide diffusion space as 40% of the gland's volume (in hypophysectomized rats) can be compared with the 22% and 27% values which we obtained for the diaphragm and the adrenal, respectively. Even a cursory examination of the thyroids in Figure 3 makes it clear that the stroma of the gland does not constitute 40% of its volume. This necessitates the assumption that the

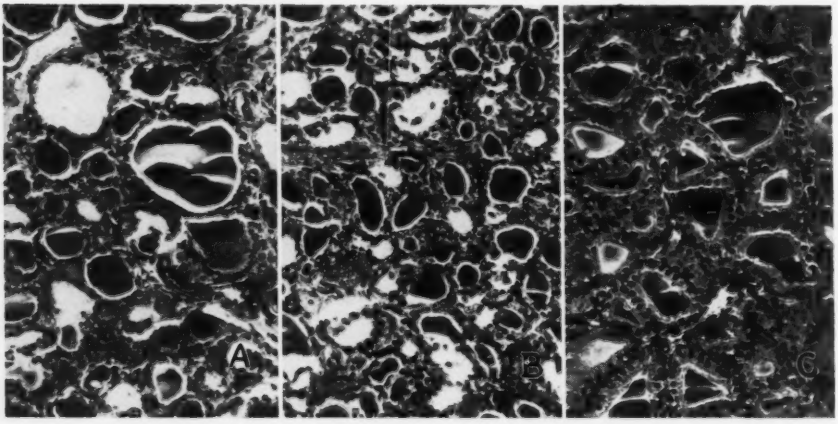


FIG. 3. Photomicrographs of thyroids. ×155. A) Thyroid of hypophysectomized rat. Note flat follicular epithelium. B)Thyroid of hypophysectomized rat which has received .25 USP unit of TSH 24 hrs. previously. The gland is indistinguishable from A). C) Thyroid of normal rat. Follicular epithelium cuboidal or low columnar.

iodide diffusion space of the thyroid includes part of the parenchyma, possibly the cellular portion, in addition to the stroma. In our estimation of "trapped" thyroidal iodide concentrations we could not take into account the possibility that TSH, at least in so far as it brings about changes in the cellularity of the gland, might affect the magnitude of the iodide diffusion space of the thyroid. This problem calls for studies with even larger amounts of iodide than those which could be administered under the conditions of our experiments.

Our results with thiocyanate are in agreement with the recent report of Wyngaarden *et al.* (1953) who found that its effect on thyroid:serum iodide gradients is quantitatively similar to that of iodide proper. Failure of previous investigators to depress the T/S below 1 with either KSCN or iodide was due to the fact that sufficiently high doses were not given.

SUMMARY

1) Thyroidal and serum iodide concentrations and their ratios were examined in hypophysectomized, normal, hypophysectomized-thyrotrophin

(TSH) treated and propylthiouracil (PTU) fed rats which received graded doses of I^{131}-labelled stable iodide following the blocking of thyroid hormone biosynthesis with a single dose of PTU.

2) Appropriate increase of the serum iodide levels caused the thyroid: serum iodide gradient to drop to 1 or below. The magnitude of the serum iodide concentration required to abolish the gradient was dependent on the state of thyrotrophic stimulation.

3) Thyroidal iodide concentrations rose progressively as serum iodide levels were elevated. When correction was made for the iodide which entered the thyroidal "iodide space" (estimated at 40% of the thyroid volume in hypophysectomized rats) by diffusion, the capacity of the gland for "trapped" iodide was found to be limited and profoundly influenced by TSH.

REFERENCES

GROSS, J. AND C. P. LEBLOND: *J. Biol. Chem.* **171**: 309. 1947.

HALMI, N. S., B. N. SPIRTOS, E. M. BOGDANOVE AND H. J. LIPNER: *Endocrinology* **52**: 19. 1953.

INGBAR, S. H. AND J. ROBERTS: *J. Clin. Endocrinol. and Metab.* **12**: 975. 1952.

JOHNSON, H. W. AND A. ALBERT: *Endocrinology* **48**: 669. 1951.

LEBLOND, C. P.: *Rev. canad. biol.* **1**: 402. 1942.

LEBLOND, C. P. AND P. SÜE: *Am. J. Physiol.* **134**: 549. 1941.

RABEN, M. S. AND E. B. ASTWOOD: *J. Clin. Investigation* **28**: 1347. 1949.

TAUROG, A., I. L. CHAIKOFF AND D. D. FELLER: *J. Biol. Chem.* **171**: 189. 1947.

VANDERLAAN, W. P. AND M. A. GREER: *Endocrinology* **47**: 36. 1950.

VANDERLAAN, J. E. AND W. P. VANDERLAAN: *Endocrinology* **40**: 403. 1947.

WALLACE, G. B. AND B. B. BRODIE: *J. Pharmacol. and Exper. Therap.* **61**: 397. 1937.

WYNGAARDEN, J. B., J. B. STANBURY AND B. RAPP: *Endocrinology* **52**: 568. 1953.

CHEMICAL FORM OF THE IODINE PRESENT IN THE HAIR OF THE RAT

C. P. LEBLOND

Department of Anatomy, McGill University, Montreal, Canada

SEVERAL authors have observed the presence of iodine in the hair of mammals (Elmer, 1938; p. 82). The quantity of iodine per 100 gm. of hair was found to be 0.108 mg. in man (von Fellenberg, 1926), from 0.008 to 0.053 mg. in the rabbit (Maurer and Ducrue, 1928), and 0.437 mg. in the albino rat (Leblond, 1951). In the last case, the concentration of iodine in hair was about 100 times greater than in other organs.

It is well known that abnormalities of the fur and particularly a pronounced retardation of hair growth occur after thyroidectomy and that normalcy can be restored by administration of thyroid preparations (Harington, 1928). It was a reasonable possibility that the thyroid hormone had to be present within the hair to produce its growth-promoting effect. The hormone would thus provide the hair iodine (by weight thyroxine is 65%, and tri-iodothyronine, 58% iodine).

The present investigation was undertaken to test this hypothesis, and more generally to determine the chemical nature of the hair iodine.

EXPERIMENTAL

The iodine estimation method used in this work was a modification of the techniques described by Chaney (1940), Barker (1948), and Gross (1949). Firstly, the distribution of iodine was examined in three adult female rats weighing 290–310 gm. These animals had been raised on Purina Fox Chow, but they were fasted for twelve hours before sacrifice to reduce possible variations due to ingestion of different amounts of food. The iodine distribution (Table 1) showed that the brain contained the least, muscle somewhat more, and other tissues such as liver, thymus, etc., a slightly greater amount of iodine. On the other hand, the hair contained approximately 100 times as much iodine as these tissues, namely, 0.375 mg. per 100 gm. (The thyroid contained 155 mg. of iodine per 100 gm., that is, several 100 times more than the hair.)

Since these animals had been fed on Purina Fox Chow—a diet containing 0.230 mg. of iodine per 100 gm. and supplying, therefore, about ten times the normal iodine requirement of the rat—their urine presumably con-

Received for publication July 23, 1953.

TABLE 1. MEAN IODINE CONTENT OF ORGANS AND TISSUES IN THREE ADULT FEMALE RATS

(mg. iodine per 100 gm. tissue)

Brain		0.001
Muscle	—cardiac	0.003
	—skeletal	0.002
Ovaries		0.005
Lymphoid tissue	—thymus	0.006
	—lymph nodes	0.004
	—spleen	0.007
Liver		0.007
Kidney		0.007
Lung		0.008
Plasma		0.008
Hair		0.375

tained a relatively large amount of iodine. In a previous investigation (Leblond, 1951) 0.039 mg. of iodine per 100 cc. of urine was observed. Inasmuch as the three animals had been housed in one cage, the hair might easily have been contaminated by the excreta. The apparent iodine content, therefore, could have been due to such contamination. This possibility was examined in a second experimental series composed of six male rats weighting from 177–256 gm. These animals were kept in individual cages which were replaced every other day. A two inch wide strip along the midline of the backs of the animals was shaved. When new hair had grown in, this area was cleansed with 1% *Dupanol*, rinsed with water, and about 50 mg. of the new hair was plucked and then analyzed for iodine. The mean iodine content of this hair, 0.402 mg. per 100 gm., was not significantly different from that observed in the animals of the first experiment. Since under these conditions the hair should not have been contaminated by urine, it was concluded that the estimated iodine was an actual component of the hair.

Thirdly, to determine the chemical nature of the hair iodine, each of the latter six animals was injected with 20 μc. of radio-iodine daily for five days. Again the hair was cleansed as described above and plucked. The hair was extracted with butyl alcohol (with or without previous softening by sodium hydroxide). The butyl alcohol extract, was evaporated to dryness and the residue chromatographed (for method, see Gross and Leblond, 1951). The chromatograms revealed that radioactivity was present[1] and was composed wholly of iodide without any thyroxine or triiodothyronine being

[1] The possibility of damage to growing hair in therapeutic use of radioiodine should be considered.

present. Thus, the hair iodine did not consist of organically bound iodine which had been taken up by the hair while exerting its hormonal effect.

Another reasonable possibility was that the effect of thyroxine on hair growth was associated with its breakdown to iodide within the hair, and that this iodide was held therein. This theory was tested by attempting to rid the animal body of thyroxine by thyroidectomy. Accordingly, in the fourth experiment, seven thyroidectomized and seven intact control male rats weighing 100–150 gm. were kept for 6 weeks after operation on a diet of Purina Fox Chow. The rate of hair growth was much slower in thyroidectomized than in control animals. However, the iodine content of the regenerating hair was the same in both groups, namely, an average of 0.399 and 0.366 mg. of iodine per 100 gm. of hair in thyroidectomized and control animals respectively. Furthermore, in both groups, chromatography of the hair after radioiodine injection revealed that the radioactivity present was again wholly in the form of iodide. It was concluded that, regardless of the amount of hair formed, the quantity of iodide therein was constant and was not influenced by the level of endogenous thyroid hormone.

Since Purina Fox Chow provides ten times the normal requirement of the rat, the high iodine content of the hair may simply reflect the high dietary intake. Normal and thyroidectomized rats were, therefore, fed Remington's low iodine diet No. 342 (1937) to which 10% yeast had been added. After two months the hair was analyzed. In the thyroidectomized group, the hair contained only 0.055 mg. of iodine per 100 gm. on the average. This small amount was not due to the absence of thyroid hormone, since an even smaller amount was found in the intact controls, namely, 0.006 mg. of iodine per 100 gm. of hair on the average. It was concluded that, in both types of animals, the low iodine concentration of the hair reflected a low level of circulating iodide resulting from the low iodine content of the diet. In intact controls, the swift uptake of iodide by the thyroid would further reduce the amount of circulating iodide available to the hair.

In conclusion, it is felt that the iodine content of the hair might be used as a practical index of the amount of circulating iodide in the rat.[2]

SUMMARY

The hair of Purina Fox Chow fed rats contains a relatively high concentration of iodine in the form of iodide. The level of iodine in the hair seems to be related to the iodine content of the diet.

---

[2] Whether clipped (1st experiment) or plucked (2nd, 3rd, and 4th experiment), the hair contained approximately the same amount of iodine. Iodide is presumably diffused throughout the entire length of the hair

Acknowledgment

This work was supported by a grant of the National Research Council of Canada. The assistance of Miss Jeanne Cambron is acknowledged.

REFERENCES

BARKER, S. B.: *J. Biol. Chem.* **173:** 715. 1948.
CHANEY, A. L.: *Ind. Eng. Chem. (Anal. Ed.)* **12:** 179. 1940.
ELMER, A. W.: Iodine metabolism and thyroid function. London. 1938.
FELLENBERG, T. v.: *Ergebn. d. Physiol.* **25:** 176. 1926.
GROSS, J.: Thesis. Doctorate in Philosophy. McGill University, 1949.
GROSS, J. AND C. P. LEBLOND: *Endocrinology* **48:** 714. 1951.
HARINGTON, C. R.: The Thyroid Gland, Its Chemistry and Physiology. London, Oxford Univ. Press. 1933.
LEBLOND, C. P.: *J. Am. Pharm. A.* **40:** 595. 1951.
MAURER, E. AND H. DUCRUE: *Biochem. Ztschr.* **193:** 356. 1928.
REMINGTON, R. E.: *J. Nutrition* **13:** 223. 1937.

NOTES AND COMMENTS

EFFECT OF PROLACTIN ON THE PHOSPHATASE ACTIVITY OF PIGEON CROP-SAC[1]

Increases in enzymatic activity and in certain cellular constituents of the pigeon crop-sac have been noted previously as accompaniments of hormonally induced hypertrophy of this organ. McShan *et al.* (1950) observed increases in succinic dehydrogenase activity and in the nucleic acids of pigeon crop glands as a result of prolactin administration. More recently Brown *et al.* (1951), studying the phosphorus metabolism of pigeon crop-sac with the aid of radiophosphorus, noted increases in the amounts as well as the specific activities of several phosphate fractions following injection of the lactogenic hormone. The present report describes the finding of relatively high concentrations of acid phosphatase in the crop-sacs of pigeons injected with prolactin.

MATERIALS AND METHODS

White Carneau pigeons of either sex weighing between 300 and 500 gm. were injected intramuscularly with a standard prolactin preparation containing 6.9 ± 0.6 I.U. per mg. The prolactin preparation used contained less than 5% each of growth hormone, adrenocorticotrophic hormone, thyroid stimulating hormone, luteinizing hormone and follicle stimulating hormone when bioassayed against highly purified preparations of the latter hormones in standard assay procedures. One group was injected with 0.5 mg. or 3.5 I.U. once daily for four days, while the second group received 2.0 mg., or 14.0 I.U. daily. A third group injected with normal saline served as controls. The pigeons were fasted overnight prior to sacrifice.

Sixteen to twenty hours after the last injection the pigeons were sacrificed by decapitation and the paired crop-sacs removed and weighed. After a brief rinsing in tap water the tissues were homogenized in a Waring blender with 20 volumes of normal saline. Aliquots were then used for the phosphatase determinations.

Phosphatase activity was determined using the method described by Huggins and Talaly (1948). In this method the amount of phenolphthalein liberated by phosphatase from a buffered solution of the colorless substrate, sodium phenolphthalein phosphate, is determined colorimetrically. Acid phosphatase activity was determined by incubating at 37° C. for one hour duplicate 0.5 ml. aliquots of the tissue homogenate with 5.0 ml. aliquots of pH 5.4 acetate buffer. Determinations of alkaline phosphatase activity were made in the same manner except that a sodium barbital buffer (pH 9.6) was used. The buffer solutions in both cases were 0.001 *M* with respect to the substrate. Immediately after the incubation period 5.0 ml. of glycine buffer (pH 11.2), containing pyrophosphate as a phosphatase inhibitor, was added. The amount of phenolphthalein liberated was then measured in an Evelyn photoelectric colorimeter at 540 mμ.

RESULTS AND DISCUSSION

No notable increases in alkaline phosphatase activity were observed following the injection of prolactin, as may be seen from Table 1, although significant increases in the crop-sac weights did occur. The lack of variation in the tissue weights of this group was due to the selection of the glands prior to the determinations in order to provide a more uniform group. The relatively high variation in the values given for alkaline phosphatase activity, as indicated by the high standard deviations, can be attributed to the small amounts of alkaline phosphatase activity found in this experiment.

Acid phosphatase activity, on the other hand, was found to increase markedly after prolactin administration. The increases appeared to be related to the dose of prolactin administered. The low dose produced more than a six-fold increase in the acid phospha-

Received for publication July 7, 1953.

[1] This work was supported in part by funds from the Dr. Wallace C. and Clara A. Abbot Memorial Fund.

TABLE 1. EFFECT OF PROLACTIN ON ALKALINE AND ACID PHOSPHATASE ACTIVITY OF PIGEON CROP-SACS

Treatment	No. of animals	Weight of crop-sac, gm.	Phos phatase activity*
		Alkaline Phosphatase	
Normal Saline	3	1.2±0.2	0.3±0.2
Prolactin, 3.5 I.U.	3	2.3±0.1	0.4±0.1
Prolactin, 14.0 I.U.	3	3.2±0.0	0.1±0.2
		Acid Phosphatase	
Normal Saline	7	1.3±0.1	0.6±0.3
Prolactin, 3.5 I.U.	9	2.1±0.7	4.0±1.8
Prolactin, 14.0 I.U.	9	3.1±1.0	7.3±1.5

* Activity expressed as μg phenolphthalein liberated per ml. per gram of wet tissue.
Values for crop-sac weight and phosphatase activity are mean values plus or minus the standard deviation.

tase activity, while the high dose produced a twelve-fold increase over that observed in the crop-sacs of the controls. Since the phosphatase activity is calculated on a per gram of tissue basis, it is evident that acid phosphatase activity can be used as a sensitive indicator of crop-sac growth. The relation between the dose of administered prolactin and the increase in acid phosphatase activity as compared with the dose vs. increase in crop-sac weight suggests that the former relationship might provide a more precise method for evaluating prolactin potency.

The fact that the prolactin preparation used was relatively low in its contamination with the known pituitary trophic hormones and the fact that the increased activity was associated with crop-sac growth, which is principally, if not entirely, under the control of prolactin (Bates and Riddle, 1939) suggest that this action of prolactin might be specific. However, the effect of other pituitary trophic hormones or of such end organ secretory products as the gonadal and adrenocortical steroids and thyroxin should be tested by making local intradermal injections of these substances over the crop-sacs, in order to determine more completely the specificity of this action of prolactin.

The marked increases in acid phosphatase activity suggest that this enzyme may be important in the growth of the gland or more directly in its role of supplying nutriment for the young, or both. It should be mentioned in this latter connection that preliminary results have indicated that the crop "milk" also contains high concentrations of acid phosphatase activity. No study was made here of the specificities, pH optima, or behavior toward metal ions of the enzyme (or enzymes) responsible for acid phosphatase activity.

SUMMARY

Intramuscular administration of prolactin to pigeons produced increases in acid phosphatase activity of the crop-sac which were proportional to the injected dose.

No significant increases in alkaline phosphatase activity of the crop-sac were observed under the conditions of the above experiment.

J. J. KOCSIS AND L. G. ABOOD[2]

Department of Pharmacology
The University of Chicago
Chicago, Illinois

[2] Present address: Illinois Neuropsychiatric Institute, The University of Illinois College of Medicine, Chicago, Illinois.

REFERENCES

BATES, R. W. AND O. RIDDLE, in E. ALLEN, C. H. DANFORTH AND E. A. DOISY: Sex and Internal Secretions, p. 1099, The Williams & Wilkins Co., Baltimore, 1939.

BROWN, R. W., D. M. WOODBURY AND G. SAYERS: *Proc. Soc. Exper. Biol. & Med.* **76:** 639. 1951.

HUGGINS, C. AND P. TALALAY: *J. Biol. Chem.* **159:** 399. 1945.

MCSHAN, W. H., J. S. DAVIS, S. W. SOUKUP AND R. K. MEYER: *Endocrinology* **47:** 274. 1950.

ACCURATE ORAL MEDICATION TO RATS WITHOUT USING THE STOMACH TUBE

At a time when medicine is paying more and more attention to psychosomatic influences, physiology often is still placing too much emphasis on accurate gadgets of measurements with disregard of the psychological status of the object to be observed, the animal.

To take the heart rate of a rat, the animal should be quiet and comfortable. If two safety pins are pushed through the skin as a pickup for the electrocardiogram, as has been described in the literature, the best instrument cannot compensate for the increased heart rate in the tortured animal. Overheating an animal to take changes in blood pressure in the tail will not yield readings of any value, even if agreement between various animals should be found. Identical errors may cause identical deviations.

It is customary to measure the effect of a hormone or other substance on the metabolic rate either after parenteral injection or after application by stomach tube. By closely observing the behavior of our animals, we suspected that these traumatic experiences might have some influence on the outcome of the experiments. On three consecutive days we therefore gave some rats injections of physiological saline; others received sugar solution by stomach tube. Three of ten injected rats showed in determinations on the fifth day increased metabolic rates of about 20%. The stomach tube evidently was more offensive; of 12 rats 8 showed metabolic increases of about 25%. The metabolic measurements were still high after one week. It seems that the treated animals were apprehensive of any handling, and that this effect lasted for the time of metabolic determination, which was carried on for about 90 minutes.

Since admixing the medication to the diet does not permit accurate dosage because of spilling and varying food intake, we devised the following method: plastic dishes, as used for water color painting, about 6 cm. in diameter with a smooth concave hollow, were provided with a rim of aluminum sheet of about 1 cm. width to prevent the animal from putting his paws into the dish. A few cubic centimeters of a mixture of evaporated milk and sugar syrup was placed into the hollow of the dishes, and these then were placed into the cages of the animals, which had been starved for at least four hours. The rats learned very quickly about this treat, and soon went eagerly after it, licking the dish clean. When treatment is intended the substance to be tested is weighed into the cups, the milk-sugar mixture—about 3 cc., is added and the substances are mixed with a small glass rod. When all is consumed, another 2 cc. is given and stirred with the same glass rod to resuspend any material that might have adhered to the rod or the surface of the

Received for publication July 10, 1953.

dish. We found in a large number of experiments that less than 1% of the rats failed to ingest the medication completely.

ARTHUR E. MEYER

Research Division
Fellows Pharmaceutical Laboratories
26 Christopher Street
New York 14, N. Y.

Acknowledgment

The help of Dr. Edwin E. Hays in extending the facilities of Armour Laboratories for the pursuit of this investigation is gratefully acknowledged.

THE EFFECT OF SOMATOTROPHIC HORMONE ON THE GROWTH OF NORMAL AND HYPOPHYSECTOMIZED GUINEA PIGS[1]

In 1921, Evans and Long demonstrated an accelerated growth in rats, treated with a simple saline extract of ox pituitary. Since that time, more highly purified preparations of pituitary growth hormone (somatotrophic hormone, STH) have been obtained by several investigators (Li *et al.*, 1944, 1945; Wilhelmi *et al.*, 1948; Raben *et al.*, 1951).

Thus far, exogenously administered STH has produced anabolic responses in rats (Evans and Long, 1921), mice (Downs, 1930), dogs (Teel and Watkins, 1929; Gaebler *et al.*, 1951), cats (Young, 1946; Milman, 1950), pigeons (Marx *et al.*, 1942), ferrets (Young, 1946), goats (Young, in press), and cows (Young, 1947; Cotes *et al.*, 1949). Other investigators found equivocal results in the monkey (Adamkiewicz and Guillemin, 1952), in the guinea pig (Silberberg, 1935), and in man (Raben *et al.*, 1952; J. S. L. Browne, in press).

Recent work, done at this Institute, failed to reveal any inhibition by STH of stress-induced catabolism in the guinea pig (Tonutti *et al.*, 1952). In the rat, on the other hand, STH has been used successfully for the prevention of the catabolic phenomena brought on by stress (Selye, 1951).

Hence it seemed of interest to examine whether STH has any growth promoting activity either in normal or in hypophysectomized guinea pigs.

MATERIALS AND METHODS

Thirty-five guinea pigs (males and females) weighing between 240–300 gm., were used, Eleven of the animals were hypophysectomized (parapharyngeal approach, ether anesthesia). two weeks prior to the initiation of the STH injections.

Five of the hypophysectomized animals served as control; the other 6 received 3.0 mg. of STH daily.

The remaining 24 intact guinea pigs were divided into four groups; of these, one was used as the control, while the others were given respectively 0.25 mg., 1.0 mg., and 3.0 mg., of STH daily.

Since the guinea pig is notoriously sensitive to thyrotrophic hormone, we employed an STH preparation (Armour and Co., Lot No. BGH 369 208) which both in the rat and in the guinea pig proved to be virtually devoid of contaminating thyrotrophic activity. Yet this same preparation proved to be highly growth promoting as judged by our own bioassays on the rat.

A saline solution of the STH was made up freshly every other day and administered

---

Received for publication November 26, 1952.

[1] This work was supported by a grant from the National Cancer Institute of Canada.

subcutaneously in two equal daily divided doses for ten consecutive days. The animals were sacrificed on the eleventh day.

The diet consisted of "purina fox chow," supplemented with large amounts of carrots, oats, hay and cabbage. Water was given *ad lib.* The room temperature was approximately 24° C.

Body weights were recorded daily throughout the entire experiment.

RESULTS

The results are summarized in Figures 1 and 2. Figure 1 shows that at all dose levels used in these experiments, STH failed to promote somatic growth in the intact guinea pig.

Figure 2 reveals that in the hypophysectomized guinea pig, STH not only failed to promote growth, but apparently caused a diminution of body-weight as compared with the non-hormone-treated hypophysectomized controls. It should be noted that in contradistinction to such animals as the mouse, rat, dog and cat, there is no immediate cessation of growth, after hypophysectomy in the guinea pig. Instead, a very slow and fluctuating increase in weight takes place over a period of several months.

We might add, that the thyroid, adrenal, liver and spleen of the STH treated hypophysectomized guinea pigs, likewise failed to show any increment in size during the period of observation. Completeness of hypophysectomy was confirmed by inspection with a magnifying glass.

DISCUSSION

It is a well-known fact that even the best STH preparations now available are singularly ineffective in causing anabolism or promoting growth in man. It may be useful to

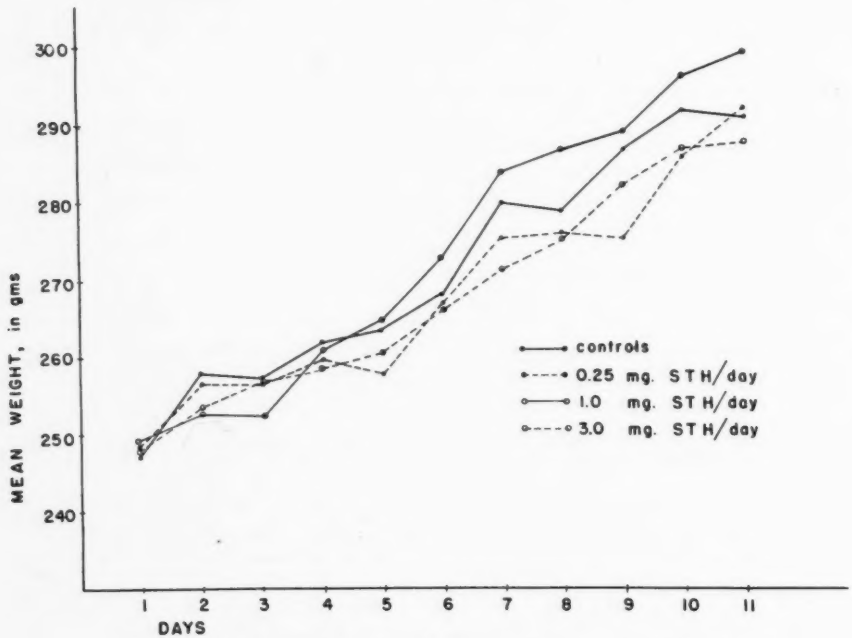


FIG. 1. Effect of STH on somatic growth of normal guinea pigs.

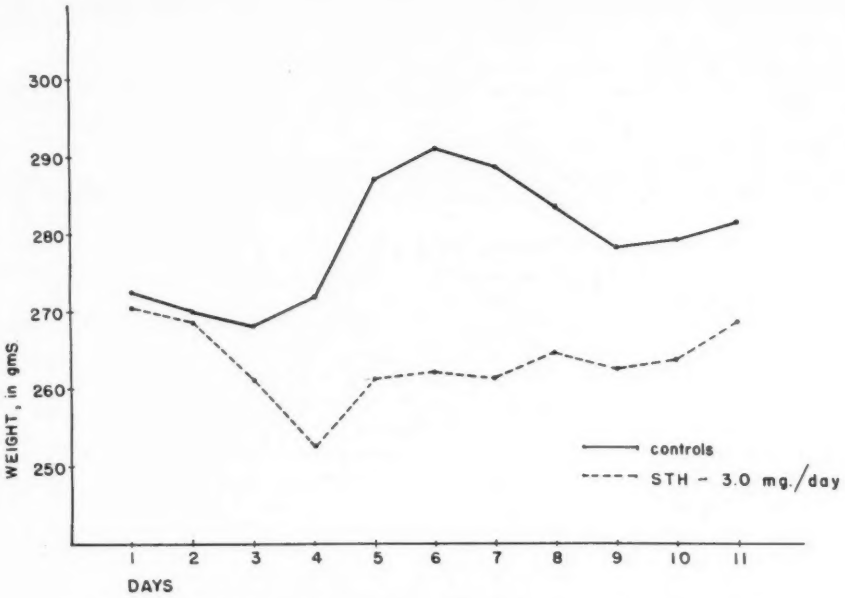


FIG. 2. Effect of STH on the somatic growth of hypophysectomized guinea pigs.

have an experimental animal which resembles man in its apparent irresponsiveness to the growth-promoting effect of STH. Since several actions of STH are largely dependent upon "conditioning factors" (e.g., diet, simultaneous treatment with steroids), the guinea pig may furnish us with a welcome experimental test object in which to explore such possibilities by large-scale experiments.

SUMMARY

Neither intact, nor hypophysectomized guinea pigs showed any somatic growth, or organ weight changes under the influence of varying doses of partially purified STH, which had proved highly active in the rat.

M. L. MITCHELL[2]
R. GUILLEMIN[3]
H. SELYE

From the Institut de Médecine et chirurgie expérimentales, Université de Montréal

REFERENCES

ADAMKIEWICZ, V. AND R. GUILLEMIN: Unpublished, 1952.
BROWNE, J. S. L.: *Recent Progress in Hormone Research.* Vol. 8. Academic Press Inc. New York, N. Y. (in press).

[2] Public Health Research Fellow of the National Heart Institute.
[3] Scholar in Medical Science.

COTES, P. M., J. A. CRICHTON, S. J. FOLLEY AND F. G. YOUNG: *Nature* **164**: 992. 1949.
DOWNS, W. G., JR.: *J. Dental Research* **10**: 601. 1930.
EVANS, H. M. AND L. A. LONG: *Anat. Record* **21**: 61. 1921.
GAEBLER, O. H., PAUL D. BARTLETT AND M. J. SWEENEY: *Am. J. Physiol.* **165**: 486. 1951.
LI, C. H. AND H. M. EVANS: *Science* **99**: 183. 1944.
LI, C. H., H. M. EVANS AND M. E. SIMPSON: *J. Biol. Chem.* **159**: 353. 1945.
MARX, W., M. E. SIMPSON, W. O. REINHARDT AND H. M. EVANS: *Am. J. Physiol.* **135**: 614. 1942.
MILMAN, A. E. AND P. DEMOOR: *Fed. Proc.* **9**: 90. 1950.
RABEN, M. S., V. W. WESTERMEYER AND A. LEAF: *Am. Soc. Clin. Investigation.* 44th Ann. Meeting. Atlantic City. 1952.
RABEN, M. S. AND V. W. WESTERMEYER: *Proc. Soc. Exper. Biol. and Med.* **78**: 550. 1951.
SELYE, H.: *Endocrinology* **49**: 197. 1951.
SILBERBERG, M.: *Proc. Soc. Exp. Biol. and Med.* **32**: 1423. 1935.
TEEL, H. M. AND O. WATKINS: *Am. J. Physiol.* **89**: 662. 1929.
TONUTTI, E., R. GUILLEMIN AND M. L. MITCHELL: Unpublished. 1952.
WILHELMI, A. E., J. B. FISHMAN AND J. A. RUSSELL: *J. Biol. Chem.* **176**: 735. 1948.
YOUNG, F. G.: *Schweiz. med. Wchnschr.* **76**: 894. 1946.
YOUNG, F. G.: *Brit. M. Bull.* **5**: 155. 1947.
YOUNG, F. G.: *Recent Progress in Hormone Research.* Vol. 8. Academic Press Inc., New York, N. Y. (1953).

EFFECTS OF PINEALECTOMY ON OVARY WEIGHT IN IMMATURE RATS

The first report that pinealectomy in the immature rat causes statistically significant ovarian hypertrophy was presented by Izawa (1926), who operated on ten 20-day-old rats of the Wistar strain. He determined ovary weights at 85 days and found the mean weight to be significantly greater than that of an equal number of control rats. This observation was recently confirmed by Simonnet, Thieblot and Melik (1951). These workers prepared three series of pinealectomized rats, each of which included 7 to 11 experimental animals; they operated at 22 days of age and examined the ovaries at 55 to 60 days.

On the other hand, several investigators have published discordant results which have engendered skepticism about the existence of a pineal-gonadal relation. Reports that pinealectomy caused no change in ovary weight have been generally accepted, despite the fact that most of these workers studied very small numbers of pinealectomized rats. For example, Kanamori's finding (1942) of no change, as determined 59 days after operation, was based on a study of only four 40- to 50-gram albino rats. Other workers used only 1 or 2 animals. The negative results of the remaining experiments are not pertinent, in that ovary weights were measured well beyond the age of normal sexual maturation. An example is afforded by the work of Renton and Rusbridge (1933), who pinealectomized 26-day-old rats and autopsied them at 340 days. Since such fragmentary or irrelevant data can hardly be regarded as valid evidence, further investigation of the problem is indicated. Accordingly, the preliminary studies described below were undertaken.

Received for publication August 25, 1953.

MATERIALS AND METHODS

Female rats of the Sprague-Dawley and Wistar strains were pinealectomized at 21, 26, or 30 days of age. Autopsy was performed at 50 days, at which time body and ovary weights were determined. The animals considered were those in which completeness of excision and absence of infection could be demonstrated. The technique of pinealectomy employed was a modification of that reported by Borell (1947). Alternate animals were sham-operated, i.e., the identical procedure was followed throughout but the pineal gland was not removed. After operation the rats were housed in an air-conditioned room in cages that accommodated 10 animals each. Pinealectomized and control animals were quartered in random order. Diet consisted of Purina Laboratory Chow and water supplied *ad libitum*.

RESULTS AND DISCUSSION

The mean body and ovary weights for the four experiments performed are presented in Table 1. The difference in mean ovary weights observed in the first series is statistically significant; it suggests that pinealectomy at 30 days causes ovarian hypertrophy at 50 days. The second and third series, in which operation was performed at 21 days,

TABLE 1. EFFECTS OF PINEALECTOMY AT VARIOUS AGES ON MEAN OVARY WEIGHT AT 50 DAYS

Series	Age at operation (days)	Experimental group	No. of rats	Ovary wt. ±S.E.* (mg.)	P	Body wt. ±S.E.* (g.)	P
I	30	Pinealectomized	8	51.2±3.2	<.01	149±3.7	>.05
		Sham-operated	8	39.8±2.0		138±5.5	
II	21	Pinealectomized	10	52.4±2.8	>.05	161±3.5	>.05
		Sham-operated	10	55.8±3.5		169±5.2	
III	21	Pinealectomized	9	48.8±4.0	>.05	147±5.1	>.05
		Sham-operated	9	39.9±4.5		143±6.7	
IV	26	Pinealectomized	32	42.6±1.4	<.01	128±2.1	>.05
		Sham-operated	32	35.7±1.6	>.05	129±2.2	>.05
		Intact control	32	36.2±1.5		125±1.9	

* Mean±Standard Error of the Mean.

Note:
Series I: Sprague-Dawley rats supplied by Holtzman Rat Co., Madison, Wisconsin.
Series II and III: Sprague-Dawley rats supplied by Charles River Breeding Laboratories, Boston, Massachusetts.
Series IV: Wistar rats supplied by Carworth Farms, New York City, New York.

yielded results that were not statistically significant, although the difference in mean ovary weights in Series III approximated that obtained in Series I. In view of these contradictory findings a fourth series was prepared; a larger number of animals was used, and an intact control group was included as a test of the nonspecific effects of surgery. Analyses of variance and covariance demonstrated (a) that the mean ovary weight of the pinealectomized group was significantly greater than that of either of the two control groups and (b) that the significance of these differences was independent of variations in body weight. No significant difference was observed between the sham-operated and the intact control groups. Histologic examination of the ovaries in this last series revealed no consistent differences among the three groups. Pinealectomy caused no significant change in body weight in any series.

Several comments are suggested by these findings. First, the ovarian hypertrophy observed was not marked; consistent demonstration of such small differences would re-

quire the use of numbers of animals so large as to minimize individual variability. Secondly, the age at operation and the duration of pineal deficiency varied among the four experiments performed. The possible significance of these various time intervals cannot be determined, since the rats used were of different strains and had been obtained from three sources.

SUMMARY

Pinealectomy in the immature rat at 26 to 30 days resulted in ovarian hypertrophy at 50 days. However, operation at 21 days produced no significant change.

Acknowledgment

This work was supported in part by grants from the Wilson Laboratories, Chicago, and the Milton Fund, Harvard Medical School, Boston.

REFERENCES

BORELL, U.: *Nord. med.* **36**: 2137. 1947.
IZAWA, Y.: *Tr. Soc. path. jap.* **16**: 72. 1926.
KANAMORI, T.: *Ōsaka kōtō igaku senmongakkō zasshi* **9**: 624. 1942.
RENTON, A. D. AND H. W. RUSBRIDGE: *Proc. Soc. Exper. Biol. & Med.* **30**: 766. 1933.
SIMONNET, H., THIEBLOT, L. AND T. MELIK: *Ann. d'endocrinol.* **12**: 202. 1951.

JULIAN I. KITAY

Biological Research Laboratories
Harvard School of Dental Medicine, Boston
and the Laboratory of Clinical Physiology
McLean Hospital, Waverley, Massachusetts

STIMULATION OF THE SECRETORY GLANDS OF THE SKIN OF THE SOUTH AFRICAN FROG (*Xenopus laevis*)

During some studies on ovulation in *Xenopus laevis* it was noted that injections of saline extracts of whole pituitary glands of a number of different species (*Rana catesbeiana, Rana pipiens*, rat, sheep, opossum, rabbit and cat) caused the release of a copious exudate from the skin. The response was characterized by a local reaction at the site of injection (dorsal lymph sac) within 30 seconds to 2 minutes and a second reaction which appeared as a generalized secretion over the dorsal area of the body, head and legs and occurred within 2 to 15 minutes after the injection. The secretion was discharged through numerous pores in the skin and formed a cloudy, white film over the animal which was eventually sloughed off into the water.

Since the extracts of whole pituitary glands appeared to cause the release of this secretion it was decided to compare the activity of a number of pituitary fractions. The results reported below are based on the minimal effective dose required to produce the generalized secretion as noted visually. In view of the fact that this response was not observed in the frog injected with saline alone, it was possible to determine threshold doses for different substances. Consequently, the response was regarded as an all or none phenomenon in these observations and the activities of the various substances was determined by using a graded series of dilutions.

Treatment with a number of pituitary preparations revealed that the activity resided

Received for publication October 24, 1953.

in the posterior pituitary. Although considerable variation in response was obtained, the minimal effective dose for pitressin was 0.005 P.U.,[1] for pitocin 0.05 I.U.[2] and pituitrin 0.005 I.U. Further tests revealed that epinephrine was effective at 0.05 μg, but that histamine (0.5 mg.) and extracts of nerve or muscle were ineffective. Epinephrine was effective *in vitro* at 1:10,000 using frog Ringer's and pieces of skin removed from the dorsum of recently killed frogs.

Histological study of the skin showed numerous poison and mucous glands surrounded by a layer of smooth muscle. Both the number of glands and the amount of smooth muscle present appeared to be greater in *Xenopus* than in *Rana pipiens* (see Holmes for description of *Rana pipiens*). Following stimulation, some of these glands could be seen with their contents either completely or partially evacuated. It is suggested that both types of glands contribute to the cutaneous, secretory reaction and that the release of the gland content is due to the contraction of the smooth muscle.

Tests on the secretion itself showed that the material is very soluble in dilute or concentrated acetic acid and insoluble in nitric acid. It is also soluble in tap water if permitted to stand for several days. Strongly positive tests were obtained with both the Millon and Hopkins-Cole reagents.

CONCLUSIONS

The release of a secretory exudate from the skin of the frog *Xenopus laevis* appears to be effected by vasopressin and epinephrine.

J. W. BASTIAN[3]
M. X. ZARROW

Department of Biological Sciences
Purdue University
Lafayette, Indiana

REFERENCE

HOLMES, S. J.: The Biology of the Frog, 1906, p. 182, The Macmillan Co., New York.

[1] Pressor Unit.
[2] International Unit.
[3] Fellow of the National Science Foundation (1952–1953).

THE 1954 ANNUAL MEETING

The Thirty-sixth Annual Meeting of The Endocrine Society will be held in the Sir Francis Drake Hotel, San Francisco, California, Thursday, Friday, and Saturday, June 17–18–19, 1954.

Dr. Roberto F. Escamilla, 655 Sutter Street, San Francisco, California, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Sir Francis Drake Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 19, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Sir Francis Drake Hotel, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. George W. Thorn, Peter Bent Brigham Hospital, Boston, Massachusetts, not later than February 1, 1954. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and Journals of the Society. The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes nor acknowledgements to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, i.e., who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, e.g., M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

Hotel reservations can be made through the San Francisco Convention and Visitors' Bureau, Room #300, Civic Auditorium, San Francisco, California. They should be accompanied by the official AMA application form which appears during December of 1953 in JAMA.

Arrangements have been made for members of this society who do not belong to the AMA to be assigned rooms during the days of the meeting of the Endocrine Society. Applications from non-members of the AMA should specify dates of arrival and departure and be accompanied by a deposit of $10 per room.

Confirmations will not be sent until after February 1, 1954, but applications should be made promptly in order to insure the reservation.

1954 AWARDS AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

FELLOWSHIPS

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

The Ayerst, McKenna and Harrison Fellowship

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949. The 1950 Fellowship was awarded to Dr. Lawrence E. Shulman and in 1952 to Dr. John C. Laidlaw.

The Fellowship will be in the amount of $5,000 and will be given on alternate years.

The Schering Fellowship in Endocrinology

This Fellowship will not be awarded in 1954 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1955.

AWARDS

The Ciba Award

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman; in 1953 the Award was presented to Drs. Sidney Roberts and Clara Szego. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than March 1, 1954.